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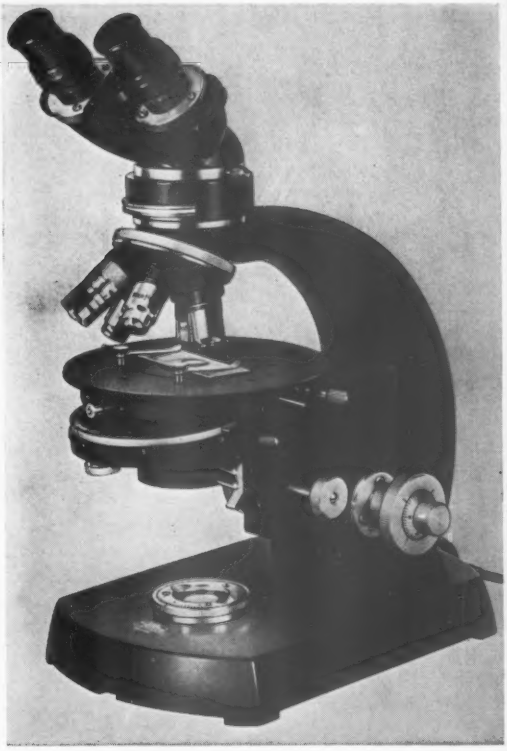

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ACTA CYTOLOGICA

THE JOURNAL OF EXFOLIATIVE CYTOLOGY

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Acta de Fundacion de la Sociedad Latino-Americana de Citologia

(Announcement of the Foundation of the Latin-American Society of Cytology)

HALLÁNDOSE reunidos en Viena, el 31 de agosto de 1961, los abajo firmantes, con motivo del I Congreso Internacional de Citología, después de haber cambiado ideas en reuniones parciales de los suscritos, en Rio de Janeiro en octubre de 1960 y en Lima en diciembre del mismo año, acordaron lo siguiente:

1° Declarar fundada en la fecha la Sociedad Latino-Americana de Citología, cuyos fines son los siguientes: Reunir a los citólogos y otros especialistas interesados en esta disciplina, de los paises latino-americanos que no tienen organizada una Sociedad local correspondiente, y agrupar a las Sociedades de Citología de los paises latino-americanos que las tuvieren, con los mismos fines científicos de la Sociedad Internacional de Citología.

2° La Sociedad estará regida provisionalmente por un Comité Directivo, integrado en la siguiente forma:

Presidente: Dra. Julieta Calderón de Laguna
Vice-Presidente: Dra. Clarice Amaral Ferreira
Tesorero: Dr. Guillermo Terzano
Secretario: Dr. Jorge Campos R. de C.
Vocal: Dra. María Rivas

y por Estatutos que serán redactados por este Comité Directivo, teniendo como base los Estatutos de las Sociedades de Citología de Mexico y Brasil; estos Estatutos serán consultados por correspondencia entre los Miembros Activos de la Sociedad y la redacción final será aprobada en la primera reunión internacional que efectue la Sociedad.

3° La afiliación a la Sociedad se hará a travez de las Sociedades de Citología de cada país de latino-america. En los paises en los que no haya tal Sociedad, y mientras se organiza una, la afiliación será directa y por invitación del Comité Directivo.

4° Se considera tres clases de Miembros: Activos, Asociados y Honorarios. Los requisitos que debe llenar un candidato para ser elegible en una de estas tres categorías, serán establecidos en los Estatutos.

5° En julio de 1962 se realizará una reunión preliminar de la Sociedad, para aprobar los Estatutos y Reglamento, reunión que se llevará a cabo durante el I Congreso Brasilero de Citología y en 1963 se realizará el I Congreso Latino-Americano de Citología, en Mexico.

6° Se acordó fijar una cuota anual de $10.00 para los Miembros Activos y de $5.00 para los miembros Asociados.

Dra. Julieta Calderón de Laguna (Mexico)
Dra. Clarice Amaral Ferreira (Brasil)
Dr. Guillermo Terzano (Argentina)
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DISEASE	NUMBER OF PATIENTS	RESULTS				INADEQUATE TRIAL
		GOOD	FAIR	TRANSIENT	FAILURE	
Lymphoma	74	34	3	5	23	9
Hodgkin's Disease	29	10	3	4	9	3
Lymphosarcoma	21	15	0	0	3	3
Multiple Myeloma	16	9	0	0	4	3
Reticulum Cell Disease	8	0	0	1	7	0
Leukemia	23	10	0	0	8	5
Chronic Lymphatic Leukemia	8	4	0	0	3	1
Acute Monoblastic Leukemia	11	5	0	0	3	3
Acute Myeloblastic Leukemia	4	1	0	0	2	1
Carcinoma (Breast, Lung, and Solid Tumors)	29	2	1	1	23	2
Miscellaneous (Mycosis, Fungoides, Psoriasis)	4	0	0	1	3	0
Total	130	46	4	7	57	16

Adapted from Wall, R. L., and Conrad, F. G.

Note that the neoplastic disorders most responsive to Cytoxan were lymphosarcoma, multiple myeloma, Hodgkin's disease, and chronic lymphatic leukemia. Occasionally, good results were observed in acute monocytic leukemia and carcinoma of the breast.

Other advantages noted in this study*

• multiple routes of administration, permitting prolonged maintenance therapy • lack of latency period for bone marrow depression • failure to produce significant thrombocytopenia • potential therapeutic effect in diseases usually unresponsive to other mustard compounds (*e.g.*, myeloma).

*Wall, R. L., and Conrad, F. G.: Arch. Int. Med. *108*:456-482, 1961.

INDICATIONS: Cytoxan is valuable for palliative therapy of certain malignant neoplasms, particularly some of those arising in the reticuloendothelial and hematopoietic systems and certain solid tumors.

Types of cancer which have proved relatively more susceptible or more resistant to Cytoxan therapy may be grouped as follows:

Group I: Neoplasms relatively susceptible to Cytoxan
- Hodgkin's disease
- Lymphomas: lymphosarcoma; giant follicular lymphoma; reticulum cell sarcoma
- Leukemia: acute; chronic
- Mycosis fungoides

Group II: Neoplasms relatively resistant to Cytoxan
- Malignant neoplasms of the breast and the ovary*
- Malignant neoplasms of the lung, the gastrointestinal tract and the genitourinary system, including the cervix and the uterus
- Malignant neoplasms of miscellaneous origin
- Malignant melanomas

*Malignant tumors of these organs are somewhat more susceptible to Cytoxan therapy than are the others included in this group.

DOSAGE: *For neoplasms relatively susceptible to Cytoxan* — Patients with lymphomas and other neoplasms believed to be relatively susceptible to Cytoxan therapy are given an initial dose of 2 to 3 mg./Kg./day intravenously. White blood counts and platelet determinations should be made daily or twice weekly and the dosage adjusted accordingly. Intravenous infusions should be continued for at least 6 days unless otherwise indicated. A leukopenia of between 1500 and 5000 cells per cu. mm. (or lower) may be expected between the tenth and fourteenth day. In the presence of a leukopenia of less than 2000/cu. mm. Cytoxan should be discontinued until the white cell count returns to 2000 to 5000 (usually within a week). Dosage is subsequently adjusted as indicated by the patient's objective response and the leukocyte count. If the patient is subjectively improved, if the size of the tumor has decreased, or if the white cells are satisfactorily maintained between 2000 and 5000/cu. mm. oral dosage may be instituted equivalent to intravenous dosage.

Thrombocytopenia is rarely observed on this regimen. If platelet counts of less than 100,000/cu. mm. are observed, the patient should be watched carefully. If platelets continue to decrease, Cytoxan should be discontinued.

The patient who has had previous treatment with alkylating agents, or x-ray, or is debilitated may be more susceptible to bone marrow depression, and initial Cytoxan doses should be more conservative than the above. Such patients should have more frequent hematologic evaluation. Good medical practice demands access to a reliable hematologic laboratory when using Cytoxan.

For neoplasms relatively resistant to Cytoxan — Patients with carcinomas and other malignant neoplasms believed to be less susceptible to Cytoxan therapy are given a dose of 4 to 8 mg./Kg./day intravenously. Unless there are indications to the contrary, this dose is continued for 6 days, then stopped. Leukopenia usually ensues on the tenth to fourteenth day after the first dose of Cytoxan. Thrombocyte reduction is not common, and platelets may actually increase. The leukocyte count promptly returns toward normal levels in most cases, and as it begins to increase, sufficient Cytoxan is administered to maintain it near 2000 to 5000/cu. mm. This may be accomplished by two intravenous injections weekly, or by oral administration, or by a combination of both routes. An oral dosage of 50 to 200 mg. daily or an intravenous injection of 5 mg./Kg. twice weekly will usually suffice.

The platelet and leukocyte counts should be followed carefully, and the prior treatment history of patients carefully evaluated as delineated above.

Leukopenia as a guide to adequacy of dosage — The best objective measure for dosage seems to be the number of circulating white blood cells. This is used as an index of the activity of the hematopoietic system, especially the bone marrow. The mechanism by which Cytoxan causes a reduction in the level of white blood cells is not known, but cessation of dosage results in an increase in the level, indicating that the hematopoietic system had not been permanently affected. When large doses (8 mg./Kg./day for 6 days) are given initially, the white cell count falls rapidly. Following the cessation of the 6-day course, the white cells may continue to decline for as long as 8 days and then increase. The reduction of the white cell count during Cytoxan therapy and its subsequent increase when therapy is discontinued can be repeated in the same patient. Maximal reduction in leukocyte count indicates the maximal permissible Cytoxan level for therapeutic effect. Leukopenic patients must be watched carefully for evidence of infection.

Total white blood cell and thrombocyte counts should be obtained 2 or more times weekly in order to evaluate therapy and to adjust dosage.

SIDE EFFECTS: Although Cytoxan is related to nitrogen mustard, it has no vesicant effect on tissue. It does not traumatize the vein when injected intravenously, nor does it cause any localized tissue reaction following extravasation. It may be administered intravenously, intramuscularly, intraperitoneally, intrapleurally or directly into the tumor, when indicated. It is apparently active by each of these routes.

Nausea and vomiting are common and depend on dose and on individual susceptibility. However, many investigators accept the nausea and vomiting in favor of maintaining maximal therapy. The vomiting can be controlled with antiemetic agents.

Alopecia is a frequent side reaction to Cytoxan therapy. It has been observed in 28% of the patients studied in this country. The incidence is greater with larger doses. The loss of hair may first be noted about the 21st day of therapy and may proceed to alopecia totalis. This effect is reversed following discontinuance of Cytoxan; during reduced maintenance therapy, hair may reappear. It is essential to advise the patient in advance concerning this effect of the drug.

Dizziness of short duration and of minor degree has occasionally been reported.

Leukopenia is an expected effect and can be used as a guide to therapy. Thrombocytopenia may occur, especially after large doses. The leukocyte or platelet counts of an occasional patient may fall precipitously after even small doses of Cytoxan, as with all alkylating agents. The drug should be discontinued in such patients and reinstituted later at lower dosage after satisfactory hematologic recovery has occurred. Prior treatment with x-ray or with other chemotherapeutic agents frequently causes an earlier or exaggerated leukopenia or thrombocytopenia after Cytoxan medication. Only rarely has there been a report of erythrocyte or hemoglobin reduction.

ADMINISTRATION: Add 5 cc. sterile water (Water for Injection, U.S.P.) to 100 mg. of Cytoxan in the sterile vial (add 10 cc. to 200 mg. vial). Shake, allow to stand until clear, remove with sterile syringe and needle and inject.

The freshly prepared solution of Cytoxan may be administered intravenously, intramuscularly, intraperitoneally, intrapleurally, or directly into the tumor. The solution should be administered promptly after being made but is satisfactory for use for three hours after preparation.

If the patient is receiving a parenteral infusion, the Cytoxan solution may be injected into the rubber tubing if the solution is glucose or saline.

No thrombosis or thrombophlebitis has been reported from injections of Cytoxan. Extravasation of the drug into the subcutaneous tissues does not result in local reactions.

PRECAUTIONS: Cytoxan should not be given to any person with a severe leukopenia, thrombocytopenia, or bone marrow infiltrated with malignant cells. It may be given with suitable precautions to patients who have had recent x-ray treatment, recent treatment with a cytotoxic agent, a surgical procedure within 2 to 3 weeks, or debilitated patients.

AVAILABILITY: Cytoxan is available as follows:

Cytoxan for Injection, 100 mg., a sterile dry-filled vial containing 100 mg. cyclophosphamide and 45 mg. sodium chloride. Packaged, 12 vials per carton.

Cytoxan for Injection, 200 mg., a sterile dry-filled vial containing 200 mg. cyclophosphamide and 90 mg. sodium chloride. Packaged, 12 vials per carton.

Cytoxan Tablets for oral administration, 50 mg., white, round tablets, flecked with blue for easy identification. Packaged, 100 tablets per bottle.

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ACTA CYTOLOGICA

Vol. 5 November-December 1961 No. 6

The Modern Concept of Respiratory Tissue Structure

FELIX D. BERTALANFFY, PH.D.

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THE concept of the structure and composition of the respiratory tissue of the mammalian lung until recently was greatly controversial. Especially the question whether the alveolar walls were actually covered by a continuous epithelium or whether the blood capillaries were in direct contact with the respiratory air was debated extensively. Only by modern electron microscope technics could this problem finally be settled.

The following is an account based upon the experience of the author and that of other workers on the newest concept of respiratory tissue structure and the histophysiology of the cells composing this tissue, with special emphasis upon their importance for exfoliative cytology. Much of the work had been performed on the lungs of rodents, and had later been reconfirmed also on other mammalian lungs. Comparative investigations have shown that there is little difference in respiratory tissue structure between mammalian species; thus, in many respects, generalizations can be made.

The *framework* of the respiratory tissue consists largely of the mucoprotein reticulin[1, 6] which is present in two forms; first as basement membranes and, second as amorphous ground or cementing substance holding together the elements of the alveolar wall. The reticular basement membranes are of two types, those supporting the capillary endothelium, the capillary basement membranes, and reticular membranes which cover as continuous sheets the surface of the alveolar walls and support the pulmonary surface epithelium. In places where capillary and surface reticular membranes meet they seem to fuse into one membrane. However, electron microscope studies have shown that in such places the two membranes remain separate.

The capillary basement membranes are laid down by the endothelial cells they support, as in blood vessels in general. The surface reticulin membrane presumably is formed by the cells of the pulmonary surface epithelium for which they serve as basement membrane. The amorphous reticulin ground substance presumably is largely elaborated by primitive, fibroblast-like cells in the alveolar wall, which at the same time are also the stem cells of the alveolar cells. Reticulin membranes are especially well demonstrated by the PA-FSA (Schiff) technic.

Normally, the respiratory tissue contains little collagen. The amounts present vary in the lungs of various animal species; thus

the bovine respiratory tissue contains more collagen than human, and this somewhat more than rat respiratory tissue. If larger than normal amounts of collagenous tissue are present, the condition is referred to as fibrosis. Investigations by the author, in which rat lungs were irradiated with large x-ray doses, showed that one factor in early fibrosis formation of the lung apparently is overproduction by alveolar cells of reticulin (pre-collagen). This subsequently matures into collagen resulting in typical pictures of lung fibrosis.

The *cellular elements* of the respiratory tissue can be divided into two categories; those which are formed in the respiratory tissue and are part of it, and cells, referred to as migratory elements, which are brought into the tissue mainly by the blood and lymph stream to be soon again extruded mainly into the alveolar spaces.

The most interesting cell, from the standpoint of histophysiology, is the so-called alveolar cell, in exfoliative cytology usually referred to as pulmonary macrophage or histiocyte. Normally, this cell occurs in three morphological forms in the alveolar wall. As primitive, fibroblast-like, diffuse cell, and as well confined cell with numerous cytoplasmic vacuoles (vacuolated) or without vacuoles (nonvacuolated alveolar cells). As it is well known, these alveolar cells are actively phagocytic, and are encountered in large numbers in most exfoliative samples of bronchial secretions and sputum. They are the representatives of the reticulo-endothelial system in the lung, thus related in phagocytic property to the Kupffer cells of the liver, reticular cells of bone marrow and spleen, littoral cells of lymph nodes, and to the diffuse macrophage system of the body in general. By fusion they give rise to foreign body giant cells and to Langhans cells of tuberculous lesions.

The alveolar cells or macrophages are formed in the alveolar walls, and phagocytose inhaled particulate matter, bacteria, etc., while still attached to the tissue. Subsequently they desquamate as fully viable, ameboid cells into the alveolar spaces, continuing their phagocytic activity. Eventually they reach the bronchioles and bronchi and are extruded with the bronchial secretions via the bronchio-tracheal tree to finally appear in the sputum. In this way they remove from the respiratory tissue large quantities of extraneous debris which otherwise with time would have impaired the respiratory activity of the lung. If excessive amounts of dust are inhaled, the alveolar cell system is incapable of keeping pace with the dust removal, resulting eventually in the well known condition of pneumoconiosis.

Vacuolated and nonvacuolated alveolar cells show somewhat different tendency toward phagocytosis. The vacuolated alveolar cells are less active and usually contain not more than some six to eight ingested granules per cell. Presumably this is associated with presence of lipid droplets in the cytoplasm, which are usually removed during histological processing rendering the cytoplasm vacuolated. The lipid material is of cholesterol nature, and it was suggested that originally it was blood cholesterol taken up by the cells while still in the alveolar wall during their close contact with blood capillaries. Thus, the vacuolated type of alveolar cells seem to be concerned primarily with removing some of the blood cholesterol.

Both types of alveolar cells, or pulmonary macrophages, are removed from the respiratory tissue in enormous numbers. Almost any smear of bronchial secretions and sputum, consisting only of a small drop, contains many hundreds of these cells. It can thus readily be imagined that the numbers of alveolar cells extruded each day via a whole sputum sample must be in the millions. One would expect that because of the high extrusion rate of alveolar macrophages the lung would soon become depleted of these cells. However, as a matter of fact, the lungs of old individuals contain

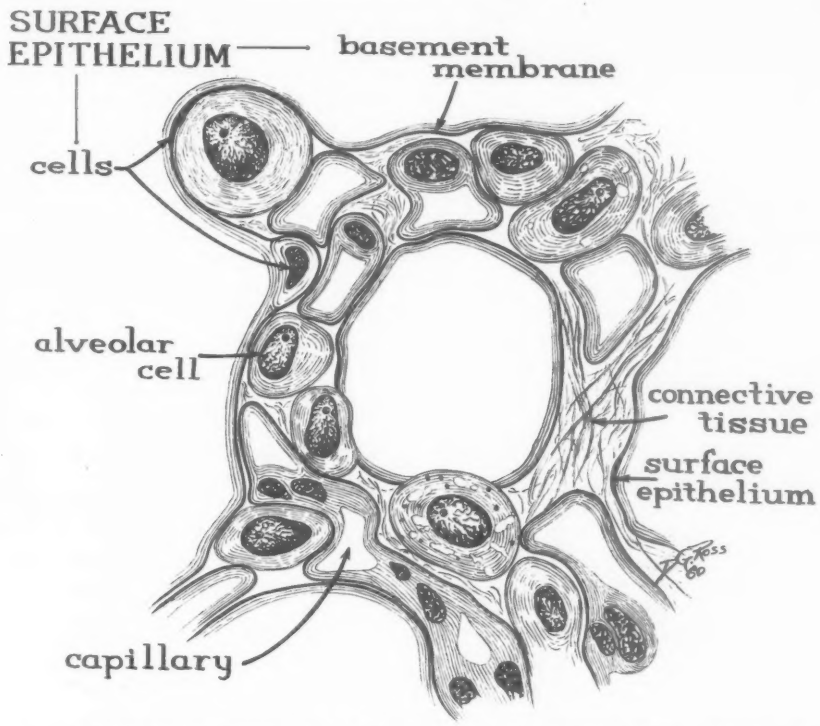

FIG. 1. *Semi-schematic reproduction of a lung alveolus.* The alveolar walls carry blood capillaries lined by endothelial cells resting on a basement membrane. In some capillaries the endothelial nuclei are visible. The cytoplasm, usually indistinct in histological sections, forms a continuous layer on the inner surface of the capillaries. In the lower portion of the alveolus is a capillary cut longitudinally to illustrate how the same capillary can appear in several sections in one plane. Between the capillaries lie alveolar cells (pulmonary macrophages) some with, others without cytoplasmic vacuoles. One of these cells in the process of desquamation pushes through the superficial reticulin membrane and possibly between two pulmonary surface epithelial cells. One of these alveolar cells contains some ingested particulate matter. The spaces between capillaries and alveolar cells are filled with fibrous connective tissue, composed mainly of reticular fibers, which form the framework of the respiratory tissue. The surface of the alveolar walls is covered by pulmonary surface epithelial cells with widely spaced nuclei and attenuated cytoplasm (which in this drawing is purposely emphasized). These cells rest on the continuous surface reticular basement membrane. Blood cells were purposely omitted.

as many alveolar cells as those of young ones. The reason for this is that by the process of cell renewal the cells extruded from the lung are as rapidly replaced by new ones formed by cell division in the alveolar walls. Both the primitive, diffuse type as well as the well defined cells, before they had ingested particulate matter, may undergo mitosis. The rate at which this cell renewal occurs was investigated by the author on the rat lung.[2, 3] It was found that in the vicinity of 12 per cent of the alveolar cells divide each day in the rat lung, implying that all alveolar cells of the lung are completely renewed once about every eight days. A very similar figure of alveolar cell renewal was obtained for the guinea pig lung, whereas it was slightly higher for the mouse lung. For technical reasons it is not possible to obtain such

figures from human lungs. However, the enormous numbers of alveolar macrophages extruded also from the human lung indicate also here a very rapid process of cell renewal possibly at rates not slower than in the rat lung. This process of cell renewal is, of course, not peculiar of alveolar cells, but also taking place in many especially epithelial structures of the body.

The second type of cells compose the recently discovered pulmonary surface epithelium. Whereas it was thought until a few years ago that the alveolar walls were bare and the capillaries in direct contact with the alveolar air, recent electron microscope investigation, especially by Low,[8, 9] Karrer,[5] and others, have established that the surface of the alveolar walls is covered by an almost continuous, endothelial-like or mesothelial-like, simple squamous epithelium. This pulmonary surface epithelium is extremely attenuated, on the average only 0.10μ in thickness,[9] and in places interrupted by an alveolar cell which, in the process of desquamation, has penetrated between the cells. The pulmonary surface epithelium rests upon the superficial reticulin membrane, which has been discovered by Leblond and Bertalanffy[6] some time before the surface epithelium was described by Low.[8] The nuclei of the pulmonary surface epithelial cells resemble morphologically those of vascular endothelium. With the light microscope, they can only be observed in carefully prepared sections stained by the PA-FSA technic demonstrating the reticulin membranes.[3] Spaced at wide distances, occasional flattened nuclei can be seen which resemble those of endothelial or mesothelial cells, and which lie external to the superficial basement membrane. The cytoplasm of these cells has no staining properties. The cells are not phagocytic, and no evidence has yet been supplied that they undergo cell renewal. If some of these cells desquamate, they cannot be recognized in exfoliative specimens. It is now believed that the pulmonary surface epithelium derived from the entodermal epithelium lining the embryonic alveoli, which sometime around term becomes extremely stretched and attenuated.

The capillary endothelial cells morphologically appear to be in no way different from those of other parts of the body. They are flattened cells with oval or oblong, darkly staining nuclei and unstained cytoplasm, which rest upon the reticular capillary basement membranes. The cells are not phagocytic and, in spite of occasional mitoses observed, are not considered to undergo cell renewal.

The second category of cells is composed of migratory lymphatic elements (lymphocytes, monocytes, and plasma cells) and granulocytes (neutrophils and acidophils) which by diapedesis have penetrated through vessel walls and entered the interstitial tissue of the alveolar walls. Similar to alveolar cells, also most of these cells eventually desquamate into the alveolar spaces to be extruded via bronchio-tracheal secretions. This process is one pathway of extrusion of leukocytes from the body; the cells thus lost are replaced by new cells formed by mitosis in lymphatic tissues and bone marrow. As compared to many other organs, the content of leukocytes in the respiratory tissue is normally high, and large numbers of these cells are continuously extruded. This explains the presence of these cells in relatively large numbers even in normal exfoliative samples from the respiratory system.

All these cellular components normally occur in certain proportions in the respiratory tissue. Cell counts yielded the following figures in rat lungs:[2] alveolar cells 31 per cent, endothelial-like cells 56 per cent, neutrophils (polymorphs) 7 per cent, eosinophiles (less than) 1 per cent, lymphocytes 5 per cent. The cells of the pulmonary surface epithelium were included with the endothelial cell count, because of the difficult recognition of these cells. During ad-

verse conditions, these cell proportions may show fluctuations. Thus in inflammatory processes, the proportion of leukocytes or pulmonary macrophages may be greatly increased both in the respiratory tissue and in exfoliative specimens.

Normal cell elements in exfoliative samples from the respiratory system are: (1) Pulmonary macrophages, alveolar cells or histiocytes, which in the course of physiological cell extrusion and renewal are removed from the respiratory tissue via the air conducting system, and thus appear in bronchial and tracheal secretions and sputum. The cells are extruded whether they had phagocytosed or not, explaining the large numbers of alveolar cells often encountered apparently not containing any ingested material.

(2) Respiratory epithelial cells of trachea, bronchi and bronchioles. Also these cells undergo cell renewal, but at slower rates. In the tracheal epithelium of the rat 2 per cent of the cells divide daily, indicating a total renewal time of about 50 days.[7] The renewal rate of the bronchial epithelium is being investigated in the author's laboratory; indications are that it is in the vicinity of that of the trachea. Apparently the basal cells (reserve cells) of the epithelium are primarily responsible for cell renewal of the ciliated cells, even though some of the latter may occasionally divide. In the small intestine, the mucous goblet cells seem to divide independently; presumably the same is also true for the mucous cells of the respiratory epithelium.

(3) Squamous epithelial cells from the oral and esophageal epithelium likewise undergo rapid cell renewal.[4] Thus in the rat, total cell renewal of the buccal epithelium requires 4.3 days, that of the tongue about six days, and the esophageal epithelium nine days. Invariably the basal cells of the stratified squamous epithelia divide by mitosis and thus supply new cells for renewal.[4]

(4) Leukocytes, in a normal pathway of extrusion, leave the vessels, enter the interstitial tissue, penetrate the superficial lining of the alveolar wall or the respiratory epithelium of bronchioles, bronchi and trachea to appear ultimately in bronchio-tracheal secretions and sputum.

Some aspects of this work were supported by a research grant from the National Research Council of Canada.

Bibliography

1. Bertalanffy, F. D., R. E. Glegg and D. Eidinger: Chemical confirmation of the abundance of reticulin in the lung. Canad. Med. Ass. J. **70:** 196 and 220, 1954.
2. Bertalanffy, F. D. and C. P. Leblond: The continuous renewal of the two types of alveolar cells in the lung of the rat. Anat. Rec. **115:** 515, 1953.
3. Bertalanffy, F. D. and C. P. Leblond: Structure of respiratory tissue. Lancet. **II:** 1365, 1955.
4. Bertalanffy, F. D.: Mitotic rates and renewal times of the digestive tract epithelia in the rat. Acta Anat. **40:** 130, 1960.
5. Karrer, H. E.: An electronmicroscopic study of the fine structure of pulmonary capillaries and alveoli of the mouse. Bull. Johns Hopkins Hosp. **98:** 65, 1956.
6. Leblond, C. P. and F. D. Bertalanffy: Reticulin membranes as the framework of alveolar lung tissue in the albino rat. Canad. Med. Ass. J. **65:** 263, 1951.
7. Leblond, C. P. and B. E. Walker: Renewal of cell populations. Physiol. Rev. **36:** 255, 1956.
8. Low, F. N. and W. C. Daniels: Electron microscopy of the rat lung. Anat. Rec. **113:** 437, 1952.
9. Low, F. N.: The pulmonary alveolar epithelium of laboratory mammals and man. Anat. Rec. **117:** 241, 1953.

A Pathognomonic Colposcopic Sign of Trichomonas Vaginalis Vaginitis

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THE diagnosis of *Trichomonas vaginalis* vaginitis is made primarily by observing the organisms directly in a wet preparation or by culture. Many times the clinical findings alone suggest the presence of this infection. In most cases there is a profuse, bubbly, yellow-grey to yellow-green purulent discharge with a foul odor. The vulva is reddened at the introitus, and the vaginal walls are injected and tender. In some instances, minute points of hemorrhage and "roughening" of the mucosa are seen, giving the vagina and portio a strawberry-like appearance. On the other hand, many women harbor the parasite with neither subjective symptoms nor clinical evidence of the disease.

Almost a quarter of a century ago Adair and Hesseltine described the histopathologic changes of the vagina and cervical portio of women with trichomonal infections. They stated that the microscopic appearance is modified not only by the pathologic process, but also by certain cyclic changes in the vaginal mucosa, which are not completely understood. According to Kessel and Gafford, either toxic or bacterial mechanisms may be responsible for the primary changes seen. Be that as it may, histopathologic findings in trichomonal infections demonstrate no pathognomic features and vary with the acuteness of the process. Inflammatory cells, consisting of polymorphonuclear leukocytes, lymphocytes, and plasma cells are found in the subepithelial connective tissue. The capillaries may become dilated and disrupted, producing tiny hemorrhages. Superficial sloughing of the epithelium may also be noted.

Koss and Wolinska have well described the changes to be found in the cytologic smear. As would be anticipated, the parasite may be noted with no cytologic abnormalities and in the presence of obvious lactobacilli. In the vast majority of clinically evident cases the smear is rich in polymorphonuclear leukocytes with occasional lymphocytes, plasma cells and histiocytes. Changes in the superficial and intermediate cells consist of perinuclear halo formation, eosinophilia, nuclear enlargement, binucleation and cytolysis. Parabasal cells are increased in number with nuclear enlargement, pyknosis and, rarely, binucleation. Cervical glandular cells show occasional and slight nuclear atypia.

The colposcopic technic[7] (magnified, binocular visualization of the portio and vagina under direct light) has also been suggested as a means of studying vaginitis. When performed systematically, it offers some aid in the diagnosis of vaginitis.[3] By means of the colposcope it is possible to study both the discharge and the surface epithelial changes. The changes are more discernible when 3 per cent aqueous acetic acid is applied. The common, non-specific colposcopic finding with trichomonal vaginitis is said to be the presence of isolated clusters of dilated capillaries, sometimes with necrosis in the center.[2, 3, 8] Michalzik has found an increase of leukoplakic change

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both by colposcopy and histology. By colposcopy he described tiny white spots ("weisse Stippchen") which, by confluence, might produce larger areas of leukoplakia. As is to be expected, the inflamed areas of squamous epithelium do not take the iodine stain.

Materials and Methods

Eighty-seven patients attending the Vaginitis Clinic of the Jefferson Medical College were studied (Table 1). All patients were evaluated by clinical examination, saline preparations of the vaginal discharge, culture for trichomonads by STS medium,[6] and by colposcopy. Fifty-four were nonwhite and 33 patients were white. Fifty of the total were pregnant, while 37 were not. Of those with *Trichomonas vaginalis* vaginitis, 16 were pregnant, and ten were nonpregnant.

The vagina and portio were inspected after inserting, without lubricant, a bivalve speculum into the vagina. After examining the discharge macroscopically and taking material for smear and culture, a 3 per cent aqueous solution of acetic acid was applied gently to the cervical portio and the surrounding vaginal walls. This solution both cleanses the surface of the portio and increases colposcopic detail. The cervix was then examined with the Moeller colposcope at 10x or 20x magnification. Punch biopsies were taken from selected areas in a few instances.

TABLE 1. *Pregnancy Status and Race of Patients Studied*

	Colored	White	Total
Nonpregnant	22	15	37
Pregnant	32	18	50
Total	54	33	87

Results

Our results are summarized in Table 2. In 20 of 26 cases of *Trichomonas vaginalis* vaginitis and in eight of ten patients harboring both trichomonads and *Candida* species simultaneously, what we believe to be a characteristic sign was observed on the squamous epithelium of the portio and on the surrounding vaginal walls. This lesion appeared as a minute red point approximately one-tenth of a millimeter in diameter, surrounded by a white areola with a total diameter four or five times that of the red area (Fig. 1). These macules appeared to be in contiguity one with the other, their numbers being apparently dependent upon the clinical severity of the disease. They were best visualized beyond the area of the transformation zone (an area characterized by the presence of columnar in squamous epithelium in the form of islands of columnar epithelium, gland openings, or nabothian cysts[7]). In 31 women without

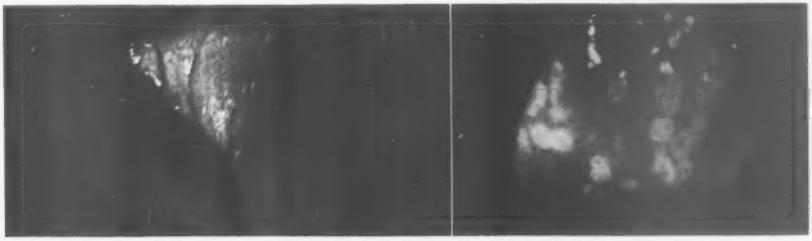

FIG. 1. Colposcopic photographs of characteristic sign of trichomonal infection. The photograph on the left (1a) shows the characteristic clustering which occurs. On the right is a cervical polyp and on the left is the vaginal wall (both out of focus). The photograph on the right (1b) is an enlargement of the sign. In actuality the central red area is approximately 0.1 mm. in diameter; this is surrounded by a white rim with a total diameter approximately 0.5 millimeter.

TABLE 2. *Summary of Results*

	Pregnant		Nonpregnant	
	Number of patients	With macules	Number of patients	With macules
Trichomonas vaginalis vaginitis	16	12	10	8
Candida vaginitis	14	0	6	0
Mixed Trichomonal and Candidal vaginitis	8	7	2	1
Other forms (atrophic, bacterial)	12	0	19	0
Total	50		37	

trichomonal vaginitis the described sign was not noted.

Three patients were examined prior to the occurrence of trichomonal infection. At this time no macules were found. With the development of trichomonal vaginitis, the above described colposcopic sign appeared. Four cases with macules present during trichomonal infections were followed after therapy. In each case the macules vanished when the infection disappeared.

White, ovoid to circular patches consisting of masses of hyphae and yeast cells and measuring from two to five millimeters in diameter could be observed grossly and colposcopically in candidal (monilial) vaginitis. These were fairly sparsely disseminated on the portio as well as on the vaginal walls. Other signs of colpitis, such as petechiae and telangiectasis, could also be visualized. In no case of pure candidiasis was the previously described sign present; the sign of trichomonal infection was found in cases of candidiasis only when concomitant trichomonal infections were present.

With respect to the whole series, in the presence of trichomonads with or without combined candidal infection, the macules were observed in 28 of 36 patients (78 per cent). Statistically, the 95 per cent confidence limits are 61 to 88 per cent.

It was not the original purpose of this project to compare statistically the occurrence and intensity of colposcopic and cytologic findings; this is another study. It has been our impression, however, that the cytologic changes are more an estimate of the severity of the trichomonal infection while the described colposcopic sign is more an indication of the presence of the parasite.

Summary

A colposcopic sign pathognomonic for trichomonal infection is described. This consists of a minute red point approximately 0.1 millimeter in diameter, surrounded by a white areola with a total diameter of approximately 0.5 millimeter. The absence of the sign is not a sure indication that trichomonads are absent but the occurrence of the sign seems to be an excellent indication that the organism is present.

References

1. Adair, F. L. and H. C. Hesseltine: Histopathology and treatment of vaginitis. I. Histopathology. Am. J. Obst. & Gynec. **32:** 1, 1936.
2. Ganse, R.: Zür Pathogenese des Portiokarzinoms und Erleichterung seiner Frühdiagnose durch farbige Kolpofotogramme. Berlin, Akademie, 1955.
3. Hinselmann, H.: Zum Ersatz des symptomatischen Fluorbegriffs durch die Krankheitsbezeichnung. Ztschr. f. Geburtsh. & Gynäk. **93:** 349, 1928.
4. Kessel, J. F. and J. A. Gafford: Observations on pathology of Trichomonas vaginitis and on vaginal implants with *Trichomonas vaginalis* and *Trichomonas Intestinalis*. Am. J. Obst. & Gynec. **39:** 1005, 1940.
5. Koss, L. G. and W. H. Wolinska: *Trichomonas vaginalis* cervicitis and its relationship to cervical cancer. A histocytological study. Cancer. **12:** 1171, 1959.
6. Kupferberg, A., R. E. Johnson and H. Sprince: Nutritional requirements of *Trichomonas vaginalis*. Proc. Soc. Exper. Biol. & Med. **67:** 304, 1948.
7. Lang, W. R.: Colposcopy. Neglected method of cervical evaluation. J.A.M.A. **166:** 893, 1958.
8. Mestwerdt, G.: Atlas der Kolposkopie, ed. 2, Jena, Germany, Gustav Fischer Verlag, 1953.
9. Michalzik, K.: Portio-Karzinom. Früdiagnose. Morphologie Genese. München. Urban & Schwarzenberg, 1959, pp. 37-38.
10. Michalzik, K.: Zur Genese des Plattenepithelkarzinoms an der Portio. Geburtsh. & Frauenh. **18:** 479, 1958.

The Significance of Parabasal ("Postnatal") Cells in the Vaginal Smear in Prolonged Pregnancy

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THE purpose of this paper is to correlate the appearance of parabasal cells in cases of prolonged pregnancy with the clinical condition of the newborn infant. Ezes,[2] Lichtfus, Pundel and Gandar[5] and others have tried to demonstrate that the appearance of parabasal or "postnatal" cells in the vaginal smear is associated with intrauterine fetal hypoxia or fetal death.

Material and Method

From 1955 to the present, 678 women with prolonged pregnancies (eight to 56 days over calculated term) have been investigated.

On the basis of our results, we divided the vaginal smear patterns into four categories: I. Late Pregnancy—onset of labor expected in ten days or more. II. Shortly Prior to Term—delivery expected within four to eight days. III. Term—onset of labor within one to five days. IV. Clearly at Term—delivery expected the same day or, at the latest, within three days of when the smear was taken.

I. Late Pregnancy: Vaginal smear pattern typical for pregnancy; navicular cells predominant, very often forming typical clusters. True intermediate cells are less in number. The ratio of the navicular cells to the true intermediate cells in this pattern approximates 3:1. Superficial cells are either quite absent or very rare. Leukocytes and mucus are absent. The epithelial cells stain strongly cyanophilic.

II. Shortly Prior to Term: This pattern differs from the preceding one mainly by the different ratio of the number of navicular to the number of true intermediate cells which is 1:1. All cells are strongly cyanophilic. The clusters of navicular cells begin to dissolve, the epithelial elements are scattered, superficial cells as well as leukocytes appear in small amounts. Mucus is usually absent. Eosinophilic index is about 2. Karyopyknotic index is about 6. Cellular borders are very distinct and sharp; the staining affinity of cytoplasm is very good.

III. At Term: The true intermediate cells are in the predominance (60-80 per cent); superficial cells with vesicular or pyknotical nuclei represent 20-40 per cent. Navicular cells comprise 5-10 per cent only. All cells in this pattern are isolated and never form the typical clusters as in the first or the second pattern. Eosinophilic index is about 8; Karyopyknotic index about 17. The number of leukocytes increases. Mucus is found in great amounts. The staining affinity of the cell diminishes.

IV. Clearly at Term: a. Regressive cell changes are in excess. Navicular cells are usually absent. In this "Clearly at Term" pattern, the superficial cells dominate (40-80 per cent). Sometimes eosinophilic, anucleate squames are seen. The staining affinity of the cytoplasm is often very low; the epithelial cells look "washed off" or "dirty." The number of leukocytes and the amount of mucus increases (in the absence of any bacterial pathological vaginal flora).

b. Mucus and leukocytes often aggregate in large clumps. Eosinophilic index is about 20; Karyopyknotic index is from 20 to 40. Sometimes, cervical cells appear in typical clusters; and, sometimes, erythrocytes are seen. If there is no mucus and only a few leukocytes and if superficial squamous eosinophilic cells with pyknotic nuclei pre-

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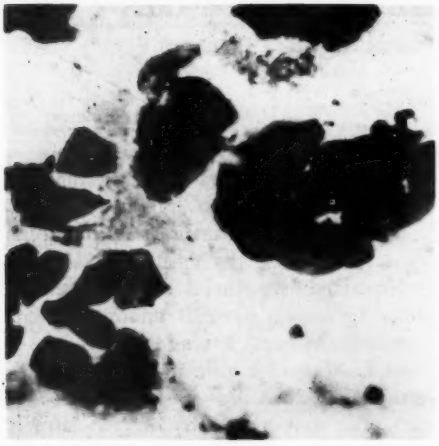

FIG. 1. Cytological pattern of "Late Pregnancy" (I).

dominate, we speak of the estrogenic pattern of the vaginal smear.

In about 12 per cent of the smears no cells can be found so that we are unable to classify the smear as one of the four patterns. The cytoplasm is completely destroyed by cytolysis, apparently because of the unusual activity of the lactobacillus vaginalis. We find only cell detritus, leukocytes and mucus. Two tablets of Trichomycin, given for two or three consecutive days are sufficient to prevent the cytolysis and in three or four days we are able to classify the smear as one of the four classes.

Results

In our series, the reliability of vaginal cytology in predicting delivery was 88 per cent. Thus, we relied upon these predictions for determining the optimal time for inducing labor. In the 180 cases where vaginal cytology was supplemented by evaluation of cervical "maturity," induction was successful in 98.8 per cent of the cases, *i.e.*, delivery of a normal infant followed induction within 24 hours.

Comparison of results using vaginal cytology with x-ray of fetal epiphyses also confirmed the value of the vaginal smear technic. The predictability of the delivery date, using both x-ray examination and vaginal cytology, was compared in 200 cases. Prediction from the vaginal smear was found to be more reliable than fetal epiphyseal x-ray in the ratio of 85:40, *i.e.*, more than twice as reliable. In addition, x-ray interpretation of fetal maturity often indicated a conservative policy, opposed to

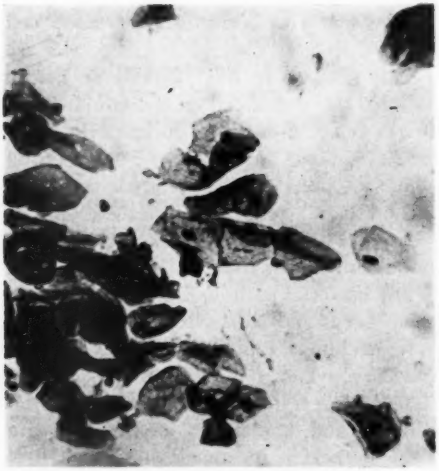

FIG. 2. Cytological pattern "Shortly Prior to Term" (II).

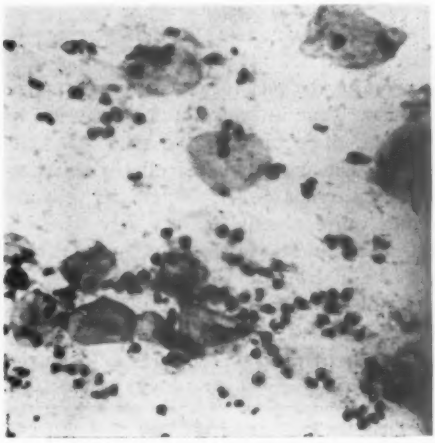

FIG. 3. Cytological pattern "At Term" (III).

induction, in cases where the fetus was already clearly suffering from placental insufficiency and subsequently born hypotrophic. In such cases vaginal cytology was found, without exception, to indicate maturity for induction and delivery.

On the other hand, the interpretation of vaginal cytology from the point of view of diagnosing pathologically prolonged gestation where the fetus is suffering from placental insufficiency is by no means clear. We agree with Watteville that no changes in vaginal cytology are specific for postmaturity.

In a series of 678 women with prolonged pregnancy, we attempted to correlate the incidence of parabasal cells in the vaginal smear with the clinical condition of the newborn infant. In contrast to Ezes and Lichtfus, we found no significant correlation whatsoever.

Parabasal cells to a varying degree were found in 83 cases of prolonged pregnancy and only once was a damaged infant subsequently delivered. When vaginal cytological findings in women who delivered traumatized infants were reviewed in detail, some interesting correlations became apparent.

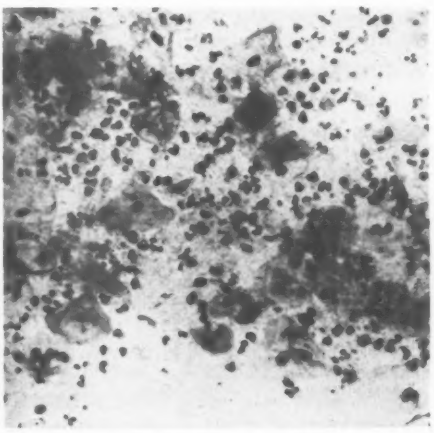

FIG. 4. Cytological pattern "Clearly at Term" (IVa).

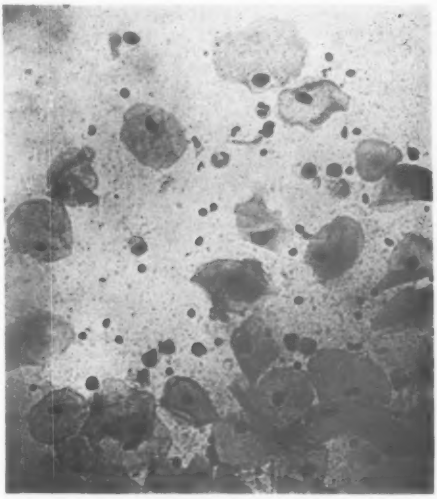

FIG. 5. Cytological pattern "Clearly at Term" (IVb).

As can be seen in Table 1, of 23 cases of stillbirth or intra-uterine birth trauma, in ten cases the cytologically determined time limit for delivery was not exceeded. The pediatrician or pathologist in no case found postmaturity as a cause of fetal death or fetal damage.

In 13 cases, where the maternal vaginal "Term" or "Clearly at Term" pattern persisted two to nine days beyond the normal limit (*i.e.*, beyond five days), the pathologist or pediatrician found no other cause of fetal damage or death than postmaturity. This group included Rh-incompatibility, congenital anomaly, mechanical birth injury, toxemia of pregnancy and infection.

In two cases, the cytodiagnosis could not be established because of cytolysis and there was not sufficient time for therapy.

It was also interesting to note that, among the infants traumatized or near death as a result of postmaturity, in only one case were parabasal cells found in the vaginal smear, although in all cases the "Term" patterns persisted for a longer than normal period prior to delivery.

All 83 cases in which parabasal cells were

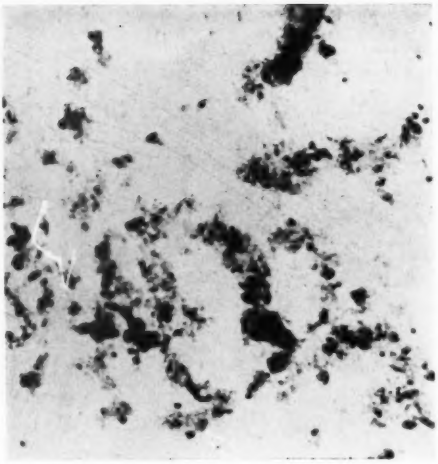

FIG. 6. Cytological pattern of "cytoloysis."

found in vaginal smears were reviewed in detail, with the following findings:

1. Pathological vaginal flora (trichomonads, mycosis) were very frequently present.

2. Cervical changes (ectropion, ectopy or erosio vera) were found particularly in the multiparae.

3. Other causes (corpora aliena, mechanical and chemical causes).

One or a combination of these findings were found in 72 of the 83 patients. In 11 women no vaginal pathology was detected in association with parabasal cells; but, on the other hand, no fetal damage occurred in this group.

TABLE 1. *Correlation of Cytodiagnosis with Fetal Trauma*
(All cases over 40 weeks of gestation age.)

Cytodiagnosis	The time limit for delivery cytologically determined: not exceeded	exceeded
I. Late Pregnancy	O	
II. Shortly before Term	O++	
III. Term	OOOO++	...*
IV. Clearly at Term		.....***

Explanation of symbols:

O traumatized due to other causes than postmaturity

\+ stillbirth due to other causes than postmaturity (congenital anomaly, preeclampsia, Rh isoimmunization, etc.)

. traumatized due to postmaturity

* stillbirth due to postmaturity

(In two cases the diagnosis could not be established because of cytolysis. In one case, the stillbirth was due to postmaturity; in the other case the newborn was traumatized due to another cause.)

TABLE 2.

Maternal vaginal smear	Clinical condition of newborn infant	Total number of cases
In all cases parabasal cells present	All healthy infants without signs of postmaturity	82
In eight cases no parabasal cells found; in one case found parabasal cells	All infants with signs of postmaturity and clinically traumatized	9
Parabasal cells not found	Postmature stillbirths	4

Discussion

One can conclude from our material, then, that parabasal "postnatal" cells in vaginal smears are a totally unreliable sign of fetal damage in prolonged gestation, but that persistence of cytological "Term" patterns beyond the cytologically determined time limit for delivery is associated with a very high incidence (12 of 13) of stillbirth or fetal damage due to postmaturity. In the one case of postmaturity occurring in the group of 83 women where parabasal cells were present, the "Clearly at Term" pattern persisted four days longer than the upper limit for the cytologically predicted date of delivery.

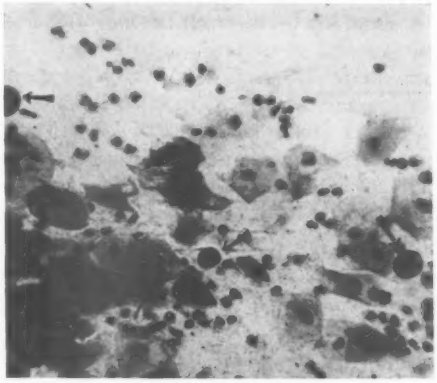

FIG. 7. Parabasal cells in the presence of pathological microbiological flora.

In a majority of the remaining 82 cases, parabasal cells appeared to be secondary to vaginal or cervical pathology rather than hypofolliculemia due to placental ageing. Our results fully confirm those of Smolka and Soost,[7] who reported that parabasal cells in the vaginal smears are associated with bacterial infection, erosion, ectropion and vaginal foreign bodies in nonpregnant women. They are also in agreement with the findings of Wolski[11] that parabasal cells appear in vaginal smears without relation to hormonal levels, purely as a result of the shedding of deeper mucosal layers in cervical erosions. Stamm[8] also considers that pathological vaginal flora may lead to the appearance of parabasal cells.

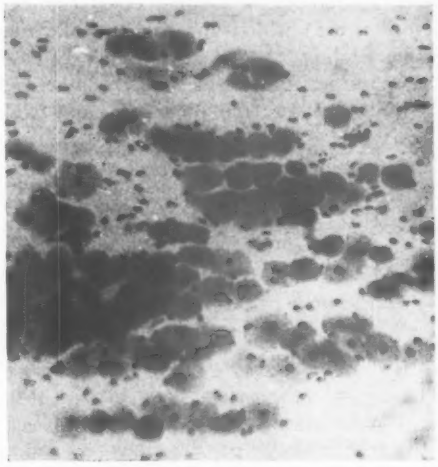

FIG. 8. Massive occurrence of parabasal cells; infant healthy.

Summary

Even though hypofollicular states may be associated with extensive exfoliation of parabasal cells, there are so many other fac-

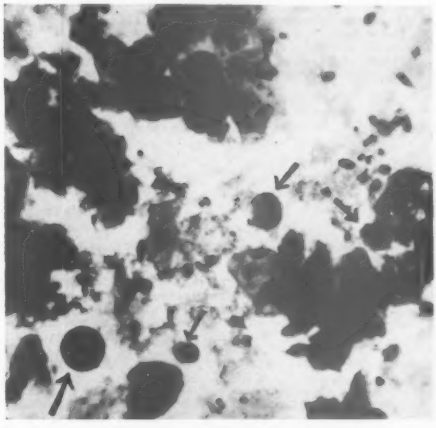

FIG. 9. Parabasal cells in the presence of ectropic changes.

tors (pathological vaginal flora, cervical erosion, etc.) which apparently evoke the same finding, that their presence in the vaginal smear of pregnancy has no practical significance for the diagnosis of fetal damage.

On the other hand, we have found that persistence of the cytological "Term" or "Clearly at Term" patterns beyond physiological time limits for delivery reliably heralds the birth of an infant traumatized to some extent by postmaturity.

Bibliography

1. Ezes, H.: Ann. Endocr. (Paris). 14: 463, 1953.
2. ——: International Congress of Obstetrics and Gynecology, Geneva, 1954.
3. Lemberg-Siegfried, S. and O. Stamm: International Congress of Obstetrics and Gynecology, Geneva, 1954.
4. ——: Geburtsh. Frauenheilk. 15: 885, 1955.
5. Lichtfus, C., J. P. Pundel and Gandar: Bull. Fed. Soc. Gyn. Obst. 9: 644, 1957.
6. ——: Gynec. et Obstet. 57: 380, 1958.
7. Smolka, H. and H. J. Soost: Gundriss und Atlas der gynäkologischen Zytodiagnostik. Stuttgart, 1956, G. Thieme Verlag.
8. Stamm, O.: Personal communication, 1959.
9. Tumova, Z. and J. Židovský: Bull. Fed. Soc. Gynec. Obstet. 2: 435, 1957.
10. Ulrych, J. and J. Židovský: Čs. Gynekol. 8: 586, 1959.
11. Wolski, A.: Ginek. Pol. 26: 365, 1955.
12. Židovsky, J. and J. Ulrych: Gynec. Obstet. 2: 277, 1960.
13. ——: Čs. Gynekol. 4: 280, 1957.
14. ——: Čs. Gynekol. 4: 929, 1958
15. ——: Akus. Gin. 2: 37, 1960.
16. ——: Zbl. Gynak. 82: 513, 1960.

STATEMENT OF THE OWNERSHIP, MANAGEMENT AND CIRCULATION REQUIRED BY THE ACT OF CONGRESS OF AUGUST 24, 1912, AS AMENDED BY THE ACTS OF MARCH 3, 1933, JULY 2, 1946, AND JUNE 11, 1960 (74 STAT. 208)

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State of Pennsylvania } ss
County of Philadelphia }

Before me, a Notary Public in and for the State and County aforesaid, personally appeared GEORGE F. STICKLEY, who, having been duly sworn according to law, deposes and says that he is Managing Editor of ACTA CYTOLOGICA, and that the following is to the best of his knowledge and belief, a true statement of the ownership, management, etc. of the above aforesaid publication for the date shown in the above caption, required by the Act of August 24, 1912, as amended by the Acts of March 3, 1933, July 2, 1946, and June 11, 1960, embodied in section 537, Postal Laws and Regulation, printed on the reverse of this form, to wit:

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4. Paragraphs 2 and 3 include, in cases where the stockholder or security holder appears upon the books of the company as trustee or in any other fiduciary relation, the name of the person or corporation for whom such trustee is acting; also the statements in the two paragraphs show the affiant's full knowledge and belief as to the circumstances and conditions under which stockholders and security holders who do not appear upon the books of the company as trustees, hold stock and securities in a capacity other than that of a bona fide owner.

5. The average number of copies of each issue of this publication sold or distributed, through the mails or otherwise, to paid subscribers during the 8 months preceding the date shown above was 1,874.

(Signed) GEORGE F. STICKLEY

Sworn to and subscribed before me this 11th day of September 1961.

KATHARINE K. STOUT
Notary Public

(Seal)

My commission expires on February 2nd, 1965.

Symposium on Probable or Possible Malignant Cervical Lesions: Carcinoma *in Situ*

I. Histology of Carcinoma *in Situ*

(Continued from September-October Issue)

Special Microscopic Studies on Histological Sections of Carcinoma *In Situ*

ALVAN G. FORAKER

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OUR SEARCH for new technics which may cast light on the problem of carcinoma *in situ*, led us to application of interference microscopy, a new technic which permits estimation of mass of various microscopic objects. We have applied this to a study of normal ectocervical mucosa and of characteristic abnormal epithelium in 20 cases each of squamous metaplasia, dysplasia, carcinoma *in situ* and invasive carcinoma of the cervix. As in our histochemical studies (a preceding topic) no findings sharply demarcated carcinoma *in situ* from either dysplasia or invasive carcinoma.

The following were determined on nuclei from midzone of normal and abnormal epithelium: refractive index, effective thickness, dry mass per unit area, mean area, mean weight. In normal and abnormal epithelium, percentage area occupied by nuclei and weight of nuclear material in the sample were computed. In general, dysplastic, intra-epithelial carcinomatous and invasive carcinomatous nuclei gave closely conforming values.[1, 2]

We have no practical experience with electron, phase, UV or fluorescence microscopy. However, it might be well to call attention to yet another special technic: soft x-ray. Fitzgerald[3, 4] has applied this to carcinoma *in situ* of the cervix. He found that *in situ* had much less mass than average normal cells, essentially due to lack of keratinization in cancer cells. There was also less mass per unit area in neoplastic than in normal epithelium.

This study confirms our results from interference microscopy, that carcinoma cells and carcinoma cell nuclei do not have greater mass than normal cells and normal cell nuclei, per unit volume.

Bibliography

1. Foraker, A. G.: Am. J. Path. **34**: 555, 1958.
2. Foraker, A. G. and J. W. Reagan: Cancer. **12**: 894, 1959.
3. Fitzgerald, P. J.: Annals N. Y. Acad. Sci. **63**: 1141, 1956.
4. Fitzgerald, P. J. and V. Yermakov: Abstracts of Papers, Seventh International Cancer Congress, London, page 385, 1958.

Discussion

Gunter F. Bahr, Stockholm, Sweden: Dr. Moberger using Roentgen absorption spectrophotometry as a method for dry weight determination in biological objects and ultraviolet absorption spectrophotometry found a decreased concentration of cytoplasmic proteins and a constant amount of nucleic acids per unit of weight in mouse vaginal squamous epithelium during the early stages of carcinogenesis. In the developed squamous cell carcinomas, significantly increased concentrations of cytoplasmic nucleic acids per unit of weight were found in the peripheral cell layers of infiltrating

cords (frozen-dried and formalin-fixed material). The same phenomena were found in developing cancers of the mouse epidermis (Acta Radiologica, Suppl., 112, 1954).

The studies of Fitzgerald are in close agreement with the above findings. I do not quite understand whether Foraker has also found a decreased mass per unit volume in dysplasia, carcinoma *in situ* or invasive carcinoma or whether their mass per unit volume is equal (do not have greater mass) to that of normal cells.

Dr. Moberger adds that despite the decreased concentration of proteins in the spinous layers of non-neoplastic proliferating epithelium and precancerous epithelium an increased volume of the spinous cells results in a considerable increase of the total amount of proteins per cell as compared with the spinous cells in normal epithelium. Taking Foraker's results on nuclei from carcinoma cells into consideration, the same will hold true for the total mass of cancer cell nuclei, namely that their mass is considerably increased. An increase in concentration of proteins (also keratinous proteins) seems to be a feature of differentiation, *viz.*, a functional change, while proliferation and cancerous dedifferentiation seems to be accompanied by an increased water intake of the cells.

Carlo Sirtori, Milano, Italy: Foraker's results deny any value of the interference microscopic technic for cancer investigations. I have no experience in this field, but I am rather surprised to learn that the interference microscope is unable to reveal the greater amount of water and thymonucleic acid in the malignant cells. Thus this technic should be considered as inadequate for cancer investigations.

Closing Remarks

Alvan G. Foraker: Since initiation of this particular "symposium by correspondence" some time ago, our principal paper on interferometry in cervical lesions has been published (Cancer. **12:** 894, 1959). Examination of this may clarify some of the questions raised.

In answer to Bahr, no significant differences in dry mass per unit area or allied properties were found in the various types of squamous cell nuclei (normal, metaplastic, dysplastic, carcinoma *in situ* or invasive carcinomatous). Since our study concerned nuclei only, the effects of keratinization in the cytoplasm did not affect our results. Visually, keratin appears to have a high mass per unit volume by interferometric microscopy, but we have not made systematic measurements of this phenomenon.

With regard to Sirtori's comments, interferometry in its usual form, permits only determination of dry mass with allied phenomena. In our studies[1] water was extracted before measurement. Even if the cells are examined in a watery medium, the water *per se* is not usually included in mass determination. Interferometry will not determine the mass of any particular cell constituent (*e.g.*, thymonucleic acid) but only the total mass present. A possible increase in thymonucleic acid in malignant cells may conceivably be accompanied by a decrease in some other substance, leaving a relatively unchanged total dry mass per unit volume. More specific approaches using interferometry can be designed to measure the mass of any substance X, by doing mass measurements before and after extraction of the substance. We do not think our results deny any value of the interference technic for cancer investigations, or general biologic studies. We believe our studies, with those of Fitzgerald and others using different approaches, have helped to eradicate a misconception which has become embedded in the literature—that cancer cell nuclei have a greater mass per unit volume than comparable normal cell nuclei. This certainly seems not to be true in uterine cervical squamous mucosa. Increased total mass depends on larger size of the cancer cell nuclei.

Interference microscopy is a valuable, albeit laborious, tool which has a definite place in biological investigation. Its range of potential and its definite limitations must be understood before it can be given optimum employment in cancer or other research.

II. Inspection Technics for Carcinoma *in Situ*

Colposcopy of Carcinoma *in Situ*

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FIGURE 1 shows protruded areas which no longer display glandular openings. Histologically one can state that strongly proliferating and increasingly atypical epithelium grows *en bloc* and fills out the glandular openings (Classes IIIb and c, after Hinselmann) (Fig. 2). Figure 3 shows whitish, slightly elevated and already exophytic appearing bizarre areas, which correspond to the former grape-like structures of the ectopy. The remainders of the ectopy showing the grape-like form can be seen directly on the external os. In cases like this the cytological reading used to be Papanicolaou Class V. The histological examination of the cone biopsy revealed a microcarcinoma in the nomenclature of Mestwerdt. In these and similar colposcopical findings of a carcinoma *in situ* it

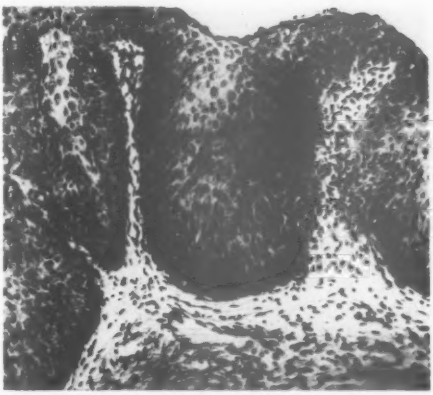

FIG. 2. Class III b and c after Hinselmann

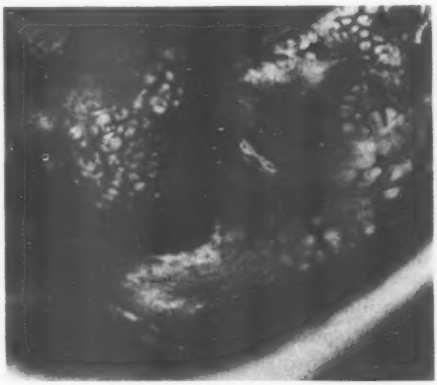

FIG. 1. Elevated "Felderung."

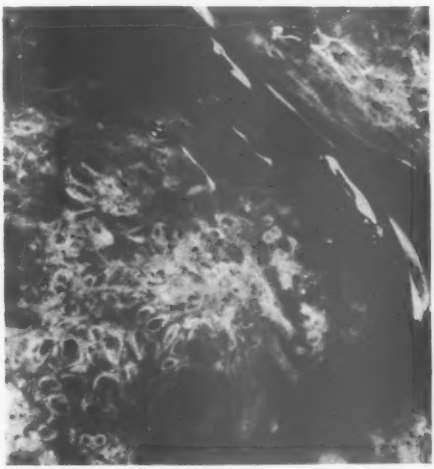

FIG. 3. Elevated, bizarre exophytic base (Felderung).

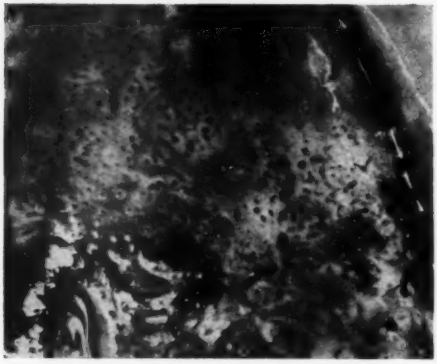

FIG. 4. Papillary "Grund" with capillaries visible in the top.

seems that together with the beginning epidermization a high degree of differentiation evolves. Besides the elevated mosaics, the horn surrounds glands which often form a leukoplakia and the bizarre-looking fields, there is also the papillary base, which usually indicates a surface carcinoma (Classes III and IV after Hinselmann). Figure 4 shows this mentioned papillary base, which is characterized by elevated papillae with a vascular loop in the top of each one. All of these mucosal alterations yield Papanicolaou Class V (cytologically). The cytological or colposcopical alterations suspicious for increased atypical epithelium or carcinoma have to be reconfirmed by a biopsy or, better, a conization with subsequent histological examination of the serial sections.

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CARCINOMA *in situ* is a controversial entity that has been studied extensively by various methods including histopathology and cytology, and less frequently by Schiller test and colposcopy. Colposcopy, although accepted and utilized in continental Europe and in South America, has found few advocates in the United States. This is somewhat surprising in view of the current, intense interest in the problem of carcinoma *in situ*. Perhaps one reason is the fact that colposcopy is a clinical method of evaluating the cervix and requires time and effort on the part of the clinician—both to learn the technic and to apply it. In our opinion, colposcopy has something to offer in the study of this particular lesion.

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For completeness, colposcopy should be defined. It consists of viewing the cervix sterescopically with a bright illumination under magnification. Usually a 10x or 20x magnification is sufficient. To improve the delineation of structures seen by colposcopy, 3 per cent aqueous acetic acid and aqueous iodine (*e.g.*, Lugol's solution) are often applied as an integral part of the colposcopic examination.

Why should the colposcope have a role in the study of carcinoma *in situ*? The answer is that with its use, changes in the overall pattern of epithelial growth can be ascertained and that colposcopic findings, which must have their basis in histologic variations, may serve to alert the clinician to histologically abnormal epithelium. The colposcope can not only detect presymptomatic carcinoma *in situ*, but can also aid

in determining its extent and its recurrence. Should the *in-situ* lesion begin in the endocervix outside the range of the colposcopic view, then one cannot detect the abnormal epithelium unless there is spread to the ectocervix.

Abnormal portio findings by colposcopy are listed below. The various leukoplakias were described in more detail in this journal: Volume 5: 115 (March-April), 1961.

1. Leukoplakia
   a. Simple (area of whitening)
   b. Mosaic (yellow-white area with mosaic appearance)
   c. Ground (yellow-white area with stippled appearance)
2. Iodine-nonstaining areas
3. Abnormal transformation zone
   (A transformation zone is a mixture of columnar in squamous epithelium in which there are islands of columnar epithelium in squamous epithelium, gland openings onto squamous epithelium and Nabothian cysts. An abnormal transformation zone is one with a glassy appearance, friability, abnormal blood vessel formations, and possibly thickened leukoplakic rims around gland openings.)
4. Proliferation
   (A characteristic of grossly visible carcinoma)
5. Ulceration or true erosion
   (Also a characteristic of grossly visible carcinoma)
6. Abnormal blood vessels
   (Adaptive vascular hypertrophy of Hinselmann) with "comma" and "corkscrew" arrangements. These often occur in combination with other abnormal colposcopic findings.

Are there any pathognomic colposcopic changes of carcinoma *in situ*? The overall view would seem to be that there are no such changes. The majority of cases demonstrates the various forms of leukoplakia, an abnormal transformation zone or perhaps a superficial true erosion.[1-4]

A frequent question of United States' physicians when confronted with the practical use of colposcopy concerns the respective roles of colposcopy and cytology in the detection of carcinoma *in situ*. Several articles comparing the methods have recently appeared.[5-8] It is the general opinion of the authors of these presentations that one method complements and supplements the other. The advantages of employing colposcopy with cytology are well outlined by Navratil, *et al.*, as follows: 1) colposcopy results in no significant loss of time, since it merely replaces the necessary speculum examination; 2) colposcopic diagnosis is immediate and arrives at its results through examination of the patient herself; 3) the desirable site of biopsy can be accurately chosen with the use of the colposcope; 4) with negative cytology, colposcopy can dispose of suspicion on the basis of clinical examination in a great number of cases; 5) colposcopy enables one to perform a target smear for cytological study of a specific area.

Bibliography

1. Lang, W. R.: J.A.M.A. 166: 893, 1958.
2. Limburg, H.: Die Frühdiagnose des Uteruscarcinoms: Histologie, Kolposcopie, Kolpophotographie, Cytologie, Biochemische Methoden, Ed. 3. Stuttgart, 1956, G. Thieme.
3. Walz, W.: Geburtsch. u. Frauenh. 18: 243, 1953.
4. Wespi, H. J.: Early Carcinoma of the Uterine Cervix, Pathogenesis and Detection (trans. by M. Schiller). New York, 1949, Grune and Stratton.
5. Lang, W. R. and A. E. Rakoff: Obst. & Gynec. 8: 312, 1956.
6. Limburg, H.: Am. J. Obst. & Gyn. 75: 1298, 1958.
7. Navratil, E., E. Burghardt, F. Bajardi and W. Nash: Am. J. Obst. & Gyn. 75: 1292, 1958.
8. Schmitt, A.: Obst. & Gyn. 13: 655, 1959.

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IT would considerably transgress the scope of this paper if I would treat all details of colposcopical diagnosis of carcinoma *in situ*. Since 1951 we have used in our clinic: colposcopy, cytology and colpomicroscopy for early detection of cervical carcinoma. For this reason we are able to indicate precisely the efficiency as well as the limits of these methods. Therefore I shall treat only the most important colposcopical findings as well as the limits of colposcopical diagnosis.

It is well known that the cervix can be viewed by aid of the colposcope with a magnifying factor of 10 to 20. The diagnosis is based on observation of blood vessels, color and differences in thickness of the epithelium, which in the case of carcinoma *in situ* can all change in particular ways. This can be the case, but must not be necessarily so, if the carcinoma has not spread too much histologically and its epithelial structure has not yet reached the particular thickness.

The following findings were described by Hinselmann as being particularly suspicious or forming at least "matrix areas" for a carcinoma *in situ*.

FIG. 1. Colpophotography of the posterior cervical lip. Extended, elevated papillary ground, sketches of irregular mosaics.

1. Leukoplakia – A leukoplakia is elevated in comparison to its normal surroundings, the surface usually being squamous and papillary. If a real cornification exists, the cornified layer can be removed easily. Below there usually are to be found atypical vessels, shaped like corkscrews (*i.e.*, Hinselmann's nomenclature "ground of leukoplakia"). Sometimes the color of such leukoplakias turns to yellow. (In that case usually no cornification exists.)

2. Ground leukoplakia—Contrary to Hinselmann's opinion, we must establish that this usually is not observed in connection with a leukoplakia. It appears much more frequently next to a leukoplakia (Table I). It is characterized by corkscrew-shaped blood vessels packed closely (Fig. 1). In the case of a carcinoma *in situ* or borders of an invasive carcinoma the ground leukoplakia is elevated – papillary appearance. The epithelium has an opaque yellowish color. The blood vessels frequently have differences in diameter.

3. Mosaic leukoplakia—This consists of areas of irregular mosaics, which originate by a network of blood vessels penetrating this area of epithelium. It is somewhat elevated compared to its surroundings and to the blood vessels throughout it (so-called "Hutchenfelderung" by Wespi). The color of these mosaics is yellowish-white. The lesions described usually do not appear singly, but rather together (Fig. 1). However, their characteristics are often not pronounced, particularly the yellowish-white color and the elevation. Quantitatively these characteristics cover a wide range. The findings mentioned usually appear with histologically benign lesions (*i.e.*, at the rim of an ectopy or a true

TABLE 1. *Colposcopical Findings of 44 Cases with Carcinoma In Situ*

Colposcopically positive findings: 28				
Ground	Leukoplakia	Mosaics	Atypical transformation zone	Tumor
3	3	2	14	1
Colposcopically negative findings: 16				
Endocervically situated, no finding at the ectocervix	Old transformation zone	New transformation zone	True erosion	
3	7	4	2	

erosion), however with the difference that they are not elevated and have no yellowish color. The diagnosis therefore must give particular attention to the color, to the appearance of the blood vessels and to the thickness of the particular area in comparison to its surroundings.

4. Atypical transformation zone (Glatthaar)—This case is most difficult for the colposcopical diagnosis, as the transitions to the benign transformation zone are rather fluent and the signs of atypias can be but sketchy. Underneath such atypical transformation zones we found the greatest number of carcinomas *in situ* (Table 1). They are characterized by a certain elevation compared to the normal surrounding, and the color turns yellowish with a strange glassy appearance (Fig. 2). The blood vessels are more winding, having occasional variations in diameter. Around the gland orifices there are yellow areas, the color of which is white in the case of benign transformation zones.

In the period 1951 to 1959 we observed 46 carcinomas *in situ*, of which 44 were examined colposcopically. The findings are listed in Table 1, each case being registered but once under its principal finding. The relatively high, 36 per cent, false negative findings can be explained by the fact that by combining all methods a carcinoma *in situ* has already been recognized at a time before corresponding blood vessels and epithelial lesions were apparent. It is interesting that 50 per cent of carcinomas *in situ* recognized by colposcopy as positive were diagnosed by the finding of an atypical transformation zone. The "old-transformation zone" caused the highest number of false negative findings. Using only colposcopical diagnosis, particular attention must be paid to this. Strangely, we found only two carcinomas *in situ* under the appearance of a true erosion, hitherto being considered as the most suspicious of all benign colposcopical findings.

It is not our intention to negatively criticize colposcopy. On the contrary, we consider colposcopy as a very important

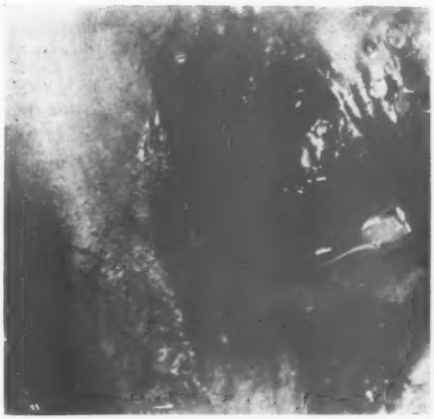

FIG. 2. Colpophotography of atypical transformation zone, predominantly on the anterior lip of cervix. The epithelium has a strange, glassy, faded appearance—little bleeding spot at 12 o'clock.

method of early diagnosis of carcinoma *in situ*. This method can be learned readily, it permits a precise localization of suspicious areas, and it simplifies the early diagnosis by eliminating immediately all clearly benign findings. It is rather our intention to indicate which findings (such as old transformation zones) should be controlled repeatedly. It is to be expected that in a carcinoma *in situ* which is slightly extended at the time of examination, it will nevertheless cause corresponding changes after a certain period.

Bibliography

1. Ganse, R.: Kolpofotogramme, Vol. 1-3. Berlin, Akademie-Verlag, 1955.
2. Glatthaar, E.: Handbuch der Biologie und Pathologie des Weibes (Seitz-Amreich). **3:** 911, 1955.
3. Hinselmann, H.: Handbuch der Gynakologie (Veit-Stockel). **6:** 854. 19??
4. Lang, W. R., A. Rakoff, F. Narvaez and G. Tartarian: Am. J. Obst. & Gyn. **74:** 1000, 1957.
5. Limburg, H.: Am. J. Obst. & Gyn. **75:** 1298, 1958.
6. Mextwerdt, G.: Atlas der Kolposkopie. Jena, Gustav Fischer, 1953.
7. Walz, W.: Geburtsh. u. Frauenhk. **18:** 243, 1958.
8. Wespi, H. J.: Die Fruherfassung des Portiokarzinoms. Basel, Benno and Schwabe, 1946.
9. Zinser, H. K. and G. Kern: Geburtsh. u. Frauenhk. **18:** 105, 1958.

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THERE are no specific colposcopical findings which may be directly related to the histological picture of carcinoma *in situ*. There are, however, a number of changes of the surface pattern of the uterine cervix, which allow for the conclusion of an atypical epithelial proliferation with a certain degree of probability and which cause the clinician to have a histological study done. In the first place the so-called "matrix areas" of Hinselmann, *i.e.*, leukoplakia, "Grund" and "Felderung," can be considered hints of that kind. In mass screenings one finds such patterns in about 6 to 8 per cent of the cases, only 10 to 20 per cent of which are reconfirmed as carcinoma *in situ* histologically. The other cases represent benign histological changes. The compulsory removal of changes of this kind, therefore, does not seem indicated to us. Rather, we have histology done only in those cases in which a "matrix area" is accompanied by a positive cytology.

Furthermore, important for the colposcopical recognition of early malignant epithelial changes is the picture called "adaptive vascular hypertrophy" by Hinselmann. It is characterized by increased formation of needle-fine and spiralled vessels or by a highly disturbing arrangement of the terminal capillary network. These vascular anomalies, only colposcopically appreciable, are very characteristic for the presence of a carcinoma *in situ* or even an invasive growth.

By means of vessel injection at the uterine cervix we have demonstrated these peculiarities of the terminal capillary network. We deal here with a typical capillary growth within a markedly proliferating papillary tissue which corresponds to an epithelium with finger-like projections into the connective tissue. That is evidence to us that there are close relationships between these colposcopically perceptible vascular anomalies in the border layer between epithelium and the connective tissue on one side and the development of malignant epithelial changes on the other.

Except for these well-defined alterations, a carcinoma can hide under irregular epidermizations. These are recognized as atypical, easily bleeding transformation zones or sites of hyperkeratotic glandular openings. Thus, there are a number of

colposcopical surface patterns which, although they cannot exactly be distinguished from physiological epithelialization processes, require cytology to decide whether we deal with a benign epidermization process, or with an atypical change. Such findings as described, which do not allow a clear colposcopical statement, can be found in about 6 to 8 per cent of all women examined.

We have gathered the colposcopical findings of 137 cytologically confirmed carcinomas *in situ*. We found that in 67.2 per cent of the cases already reviewed, the colposcopical examination raised the suspicion of an atypical epithelium; 45.3 per cent of these carcinomas *in situ* have been uncovered already at the matrix fields and the rest, 21.9 per cent, could be recognized by other auxiliary signs, *e.g.*, atypical transformation. However, 32.8 per cent of the carcinomas *in situ* could only be demonstrated by means of cytology. In one third of our material we have not been able to detect any clue for an atypical epithelial proliferation. Contained in this latter group are mainly the endocervically located carcinomas *in situ*, which are much more frequent than previously assumed.

Discussion

Jules-André Bret and Fernand J. Coupez, Paris, France: We agree with the signs of "suspicion of carcinoma *in situ*" as given by Ganse, Lang, Walz and Zinser. We also recognize that colposcopy should be limited to the tracing of abnormal zones, which then have to be biopsied or treated if cytology is negative.

Unfortunately the four papers give the impression that there is no common concept as to the definition of the colposcopical pictures, and a percentage of error is given which is much higher than the figures usually accepted.

1. Lang, whose nomenclature is particularly clear, does not give us the important difference, which he surely knows, between the aspects of "mosaic," a "base" and a histologically benign leukoplakia on one hand and the pictures of those lesions that correspond to a carcinoma *in situ on the other*. (See Bret, A. J. and F. J. Coupez: Colposcopy. Paris, Masson, 1960.)

We demonstrate that the age of the lesion may be recognized colposcopically and that with its association with the other elements it will permit differentiation between persistent lesions and those which primarily or secondarily progress. The acetic acid test and the intensity of the pictures help very much in this differentiation. It is during the process of epithelialization of these layers that these differences are most perceivable.

2. Walz lists, in his Table 1, eleven negative colposcopical findings which are classified as "old transformation zone" and "new transformation zone." Since he mentions elsewhere the existence of pictures classified as "atypical transformation zone," we are compelled to admit that certain carcinomas *in situ*, according to author, appear under the colposcope as "normal transformation zones." That means they would appear under the classical picture of an ectopy, intermingled with large amounts of normal squamous epithelium with normal glandular openings, Nabothian cysts and increased, but normal, vascularization. We who consider colposcopy as capable of very early recognition of the presence of abnormal epithelium, be it simple atypia or aggravated, cannot accept this proposition.

When we also read that, for Walz, the "true erosions" are very suspicious, whereas we consider them as usually benign (or inflammatory or traumatic origin), it becomes clear that we do not talk the same language and that these contradictions are due to a lack of common definition of the terms applied.

3. Zinser gives very interesting, exact descriptions of the vascular alterations which he studied and which are certainly a very important additional sign in the search for carcinoma *in situ*. However, we are very surprised by his 32.8 per cent negative colposcopies. In our experience the pure endocervical localization of a lesion is very rare and almost always accompanied by alterations of the "transitional zone" (Squamo-columnar junction) which attracts attention. As far as the carcinomatous lesions of the ectocervix are concerned, we do not know of a single one which colposcopically displays normal squamous epithelium.

Conclusions: The diagnostic possibilities of colposcopy have not been rationally explored and moreover they suffer from the complete lack of a common terminology. This state of confusion is very disadvantageous for the development of the method and demands immediate communications between the colposcopists of the various countries.

These communications should aim for two goals:

1. To set up a complete nomenclature of the pictures observed with their exact definitions.
2. A definition of the "colposcopical complexes" which would render a classification useful for the practitioner in his practical conclusions.

We personally hope that a colposcopical examination will always be able to give one of the three following conclusions:

a. Benign lesions: to be treated immediately after cytological study of the corpus uteri, tubes and ovaries.

b. Doubtful lesions: to be treated only after the result of cytology is known.

c. Suspicious lesions: they require immediate cytological examination and guided biopsy.

Unless such an aim is realized we believe that colposcopy will have a hard time in occupying its well-deserved place side by side with cytology in the detection and prevention of carcinomatous lesions of the uterine cervix.

Clarice do Amaral Ferreira, Rio de Janeiro, Brazil: To evaluate the value of colposcopy in carcinoma *in situ*, J. P. Rieper, Head of the Department of Colposcopy, was asked his opinion. These are his words:

"First we must explain what is classified as carcinoma *in situ*. Distinction should be made between the provisional diagnosis which is made by biopsy and the definitive one which is provided by the complete histologic diagnosis, using serial sections. Only the latter cases should be called carcinoma *in situ*, but it is common in literature to also include the cases mentioned first.

"From 10,000 patients observed in Ambulatorio Preventivo do Cancer Genital do Instituto de Ginecologia, we have found 60 cases of carcinoma *in situ* by the use of cytology, colposcopy and biopsy.

"The colposcope has revealed aspects of matrix, leukoplakia, ground, mosaic (Felderung), atypical vascularization, or atypical transformation zone in 54 cases. In the remaining six cases, it showed colpitis in five and normal epithelium in one case. Colposcopy has therefore failed in 10 per cent of the cases. These cases were located intracervically. The variable amount of experience of the several examiners should also be considered in the evaluation of the procedure.

"The most important fact to be stressed is that colposcopy covers the majority of the cytological failures and in addition can aid in obtaining the biopsy, by demonstrating the area where the tissue alterations are most severe."

Herbert Janisch, Vienna, Austria: The unusual transformation zone with its usually increased vascularized epithelium and its variances in transparency due to increased epithelia certainly represents an important colposcopical finding. It permits one almost always to suspect a malignant epithelial proliferation at the uterine cervix. There is special significance in the atypical vascular picture with its different variants (comma-, needle- and corkscrew-shaped vessels). The latter are almost always considered as the expression of rapidly occurring proliferative processes with increased blood requirements, as is especially the case in malignancy. However, the differentiation from a normal transformation zone with a highly inflammatory stromal reaction may be extremely difficult at times. Here we have clearly reached the limit of colposcopical possibilities, regardless of the experience of the individual observer.

We agree completely with Ganse, Lang, Walz and Zinser. We also have found among these colposcopical pictures the highest percentage of malignant changes, and we have almost always received histological confirmation of our colposcopical findings. The significance of the so-called "matrix areas" with their marked gradual differences have only limited value for the colposcopical detection of a marked atypical epithelium, since histologically one can detect only 10 to 15 per cent of the cases of carcinoma *in situ* in these "matrix areas." However, the experience of the colposcopist will keep the false positive and false negative results within reasonable limits.

We emphasize, as do the main authors, that colposcopical pictures can only raise the suspicion of a certain diagnosis, which subsequently must be confirmed by histology. Carcinomas located solely on the endocervix escape colposcopical detection, especially in the early stages. Until now we do not have a safe criterion for the recognition of the so-called preclinical carcinoma. This holds true for the early morphological detection methods (cytology, colpomiscroscopy) as well as for the colposcopical inspection of the cervical surface. We believe that the modern methods in use today for the early detection of carcinoma of the uterine cervix do not give us complete accuracy in diagnosis, but that the accuracy increases as more methods are jointly used.

Jacques Jenny and Alfred Wacek, Zürich, Switzerland: In our hospital we pay a great deal of attention to "extended colposcopy." By this we mean a careful inspection of the uterine cervix after applying acetic acid solution and subsequent staining with iodine. By proceeding in this manner suspicious areas, which otherwise would have remained colposcopically invisible, often become visible. Moreover, for visualization of the cervix we use the Grave's speculum, since with this instrument the external os is made to gape, thus making possible a better inspection of the region of the squamocolumnar junction. In colposcopically suspicious cases we take a guided spoon biopsy proceeding radially from the external os. Our conception of "colposcopically positive" is broader than that of Walz. Everything that is beyond the normal or atrophic cervical epithelium, the simple ectopy, and the normal old or fresh transformation zone with blurred borders after painting with iodine, is considered colposcopically suspicious. At least to us these findings are an indication for taking a Schiller surface biopsy. We have compiled 100 unselected cases of carcinoma *in situ* and plotted the alterations observed. For explanation we refer to the contributions by Walz and Lang. The left column indicates the number of cases in which the

TABLE 1.

Colposcopical findings		Total no. of colposcopical findings	Main colposcopical findings
Bleeding ectopy and transformation zone		18	10
Transformation zone with increased vascular pattern		10	2
Sharply defined transformation zone		46	24
Unusual transformation zone		29	23
glassy, opaque, whitish areas	18		
areas with turbulent vascular pattern	5		
hyperkeratotic gland openings	6		
Matrix areas		42	30
Leukoplakia	26		
"Grund"	20		
Mosaic	10		
Uncharacteristic, sharply outlined iodine negative area		18	3
True erosion		6	3
Ulcers		3	1
Polyps		2	—
Tumors		1	1
Unsuspicious atrophic cervix uteri		1	1
Unsuspicious ectopy and transformation zone		2	2

alteration indicates the total number of alterations observed. The difference in the figures stems from the fact that often one cervix shows more than one pathological finding (Table 1).

According to our results, the changes called "matrix areas" by Hinselmann, *i.e.*, leukoplakia, "Grund," "Felderung," are most common (30 per cent of the cases). The sharply defined transformation zones are found in 24 per cent of the cases. The atypical transformation zones occur in 23 per cent of the cases. In order to avoid confusion with the histological conception of atypical epithelium, they have recently been called "unusual" transformation zones. In 10 per cent of the cases a bleeding ectopy or transformation zone is the main finding. In some of these cases we are probably dealing with the misinterpretations of the examiner, and these cases probably should have been classified also as "unusual" transformation zones. The vulnerability combined with high bleeding tendency (as a consequence of increased vascularity) is here the indication for biopsy, as well as in other cases of transformation zones with a usually increased (but not turbulent) vascular pattern. It is possible that the decision was made more difficult by the concomitant bleeding, and therefore the diagnosis was made of merely an ectopy or a transformation zone. The uncharacteristic, sharply outlined, iodine negative areas occurred as the main finding in 3 per cent of the cases. We understand this term to mean changes which are not disclosed by simple colposcopical inspection or after application of acetic acid, but remain unstained with iodine and display sharply defined borders. Ulcers are found in 1 per cent of the cases, as also are tumors. In agreement with Walz the incidence of true erosion amounts to only 3 per cent of the cases.

Interesting and still not completely understood is the remarkable discrepancy in the occurrence of the colposcopically negative endocervical carcinoma *in situ*. Zinser reports the extraordinarily high figure of well over 30 per cent, whereas we have found only 2.5 per cent to be endocervical carcinomas *in situ*.[1] A similar figure is given by Limburg.[2] We are in the process of rechecking this question in our hospital. Part of the differences possibly originate from the relatively liberal interpretation of what constitutes colposcopically positive results. In addition, a purely technical difference in visualization of the cervix may be considered. With our method of inspection, we often visualize the lower quarter or fifth of the uterine cervix. Moreover, we consider "extended colposcopy" indispensable for the definitive evaluation of the cervix.

In summary, we would like to emphasize the high dependability of colposcopy in the detection of carcinoma *in situ* of the cervix, as long as the examination is done by an experienced person, and the possibility of taking guided biopsies is used when necessary. Indeed, there is no colposcopical picture specific for a carcinoma *in situ*, a fact which we would also like to emphasize. In any case, the definite diagnosis is made by histology. A coarse "Grund" and a coarse "mosaic" have to be con-

sidered with increased suspicion. To what extent the Schiller surface biopsy may be replaced by cytology in this early detection, we cannot as yet judge. We are engaged in checking this question at the present time. As far as we are able to determine from the available results, this should be quite possible.

Bibliography

1. Held, E.: Arch. Gyn. **188**: 376, 1957. Schweiz. med. Wschr. **89**: 69, 1959.
2. Limburg, H.: Stuttgart, G. Thieme, 1956.

Ernst-Helmut Krüger, Halle/Saale, Germany: When Zinser and Walz report colposcopical failures in more than 33 per cent of the cases of carcinoma *in situ*, this is an unbearable colposcopical range of error. In our experience, 90 per cent of the patients in whom later a so-called carcinoma *in situ* is diagnosed show histologically colposcopical signs on the ectocervix. This holds true also in cases where the carcinoma *in situ* is mainly located in the endocervix, the incidence of which, however, we consider to be very low. These endocervical carcinomas *in situ* are, in our experience, always accompanied by appreciable colposcopical signs on those parts of the cervix which can be visualized.

I agree with Zinser that there are no safe colposcopical indications for the presence of a carcinoma *in situ* except for the unusual abundance of vascularization. I also agree that carcinoma *in situ* presents itself in more than 40 per cent of the cases under the colposcopical picture of an "unusual transformation zone," which is very variable in its manifestations and therefore hard to define. It is not always abundantly vascularized, elevated and sharply defined toward the healthy surroundings. Whitish outlined glandular openings are, in our opinion, not exclusively characteristic for the unusual transformation zone.

We believe that the colposcopically visible vascular features, especially when they are unresponsive to hormonal stimuli and constant in their caliber, allow one within limits to draw certain conclusions as to the degree of the atypia and its growth rate.

Among the colposcopically suspicious findings are: the elevated base ("erhabener Grund"), the elevated mosaic ("erhabene Felderung"), corkscrew capillaries, needle-like capillaries ("Haarnadelgefaesse"), and tangled vessels in non-inflamed surroundings exhibiting level differences and a yellowish color of the mucosa.

There are gradual transitions between the base ("Grund") and the mosaic ("Felderung") of the various qualities. The elevated base ("Grund") however has always a higher growth rate than the elevated mosaic ("Felderung") and therefore is to be considered prognostically worse. Hence, when an elevated base ("Grund") is the finding, even with a negative cytological smear, the histological examination of the entire tissue involved should be done.

The Schiller test fails in the search for the so-called carcinoma *in situ*. We do not attribute any significance to the iodine negative, colposcopically uncharacteristic areas or to the true erosion without remarkable colposcopical findings.

The carcinoma *in situ* can be detected by means of colposcopy with the same degree of safety, but more economically than with cytology. We think, however, that one should stop opposing the two methods, since everyone will have better results with the method with which he is more familiar.

We do a routine colposcopic examination and in doubtful cases we consult the cytological specimen. We are content with the results achived in this way.

Marco Marcov, Stara-Zagora, Bulgaria: Although cytology has spread more and more, colposcopy has not only held its own position but has itself attracted more attention. Together with histopathology and cytodiagnosis it represents an indispensable part of the early clinical diagnosis of the carcinoma of the uterine cervix.

Colposcopical findings are not specific, but they reveal, in most cases, certain characteristic aspects. The relative increase of the nuclear substance (which goes together with the occurrence of cytological atypia) decreases the transparency of the epithelium which results colposcopically in an opaque or a glassy-yellowish appearance.

As a consequence of increased vascularity and hyperemia of the capillary system there is an increase in the number of papillae containing vessels. However, inflammatory vascular reactions may produce a similar appearance.

I would like to emphasize that among all the characteristic changes of the carcinoma *in situ*, leukoplakia is the least significant since the epithelial layers are covered by the superficial parakeratosis and keratinized layers.

Closing Remarks

Robert Ganse: To Bret and Coupez: We agree with Bret and Coupez insofar as colposcopy is able to recognize the presence of a typical epithelium and mucosal changes on the surface of the ectocervix in the very early stages. As far as their nomenclature is concerned, we are of the opinion that it would be very advantageous if used uniformly. Misunderstandings always occur when new terms are introduced. Contacts between the colposcopists of all countries, as recommended by Bret and Coupez, are very much welcomed. Since we ourselves have been using the terminology of Hinselmann, the complaint is aimed at the founder of colposcopy. As far as the colposcopical pictures are concerned, it is obvious that it is impossible to present all the colposcopical variations, well known to us, on four pages, however. The terminology should be uniform and in this we agree with

Bret and Coupez. It also should be rendered more precise insofar as distinguishing clearer lesions which require therapy from those which only require repeated checkups is concerned.

To Clarice do Amaral Ferreira: This discussion finds our agreement insofar as it is stated that colposcopy in the majority of cases eliminates the errors of cytology.

To Herbert Janisch: Janisch is correct insofar as he points to vascular changes. However, the slightest atypical vascular change does not belong to the colposcopical pattern of the transformation zone.

To Jenny and Wacek: We do not consider that Schiller's scraping is adequate for the following reasons:

1. The final histological diagnosis on an ectocervix which has been previously scraped and injured is rendered more difficult.

2. The histological preparation of the strips of epithelium is not easier technically than is the cytological workup. We agree approximately with the endocervical localization (4.1 per cent) as given by the authors.

To Krüger: We agree insofar as we too consider it wrong to oppose the two methods, colposcopy and cytology. Only by colposcopy—since we lack here cytological laboratories—is an early diagnosis possible on a broader scale. Using only this colposcopical method one cannot imagine what occurs endocervically. In order to bring about scientific clarity and not to oppose the two methods we use them both at our hospital.

We agree with the majority of the discussers that the terminology has to be created uniformly and also that by no means can the value of colposcopy be questioned. However, our opinion is not to change the terminology as devised by Hinselmann but to broaden it. In many cases, for instance, the abnormal transformation zone could be designated more precisely by characterizing the definitions.

Warren R. Lang: The Papanicolaou classification of cytologic smears to indicate the possibility of cancer (Classes I through V), subjective as it is, represents a welcome step forward in predicting malignancy. Unfortunately, such an approach has not yet been outlined for colposcopic changes. This may well be a main reason for variation of "positive" colposcopic findings with carcinoma *in situ*.

We concur with Bret and Coupez that a normal transformation zone in the overwhelming majority of cases indicates normal epithelium; however, since this is histologically representative of epidermidalization (or whatever other terminology is preferred) atypical squamous cells can be exfoliated. An area of true erosion, demonstrating merely loss and separation of epithelium, and an area of iodine-nonstaining of otherwise normal-appearing squamous epithelium have not been very significant in our colposcopic experience.

The controversy as to whether carcinoma *in situ* is located primarily on the portio or in the endocervical canal is a baffling one. Undoubtedly the average biopsy demonstrating an *in situ* lesion is taken from the ectocervix. The columnar epithelium, which is so often found on the portio, may confuse the pathologist who so frequently and automatically labels the specimen as "endocervical." Undoubtedly the endocervical origin is the predominantly popular concept at present.

Colposcopy, like cytology, demands interest, training and experience before one attains effectiveness in its application. In the nature of things it must miss early carcinoma just as cytology occasionally does; but as Janisch and Krüger state, the more methods utilized, the greater chance there is of detection of malignancy in its incipiency.

Wolfgang Walz: It was to be expected that especially in the discussion of the colposcopical diagnosis of carcinoma *in situ* the opinions would be divergent. In my own view, these differences of opinion stem mainly from the fact that the various investigators perform the detection of early carcinoma in various ways. There is a difference whether one uses colposcopy alone or one combines colposcopy with cytology and colpomicroscopy.

To Bret and Coupez: Apparently my statements have been misunderstood, since I too certainly consider the true erosion a benign lesion. I have merely designated it as the colposcopically most suspicious of the benign lesions. This has been done for a good reason, since often this lesion is considered a condition which colposcopically cannot be unequivocally clarified. I intended in my statements to oppose just this very erroneous assumption. In this context I may refer to the discussion of Wacek and Jenny, who draw the circle of the colposcopically suspicious changes larger than I do. Without doubt, one is compelled to do so in cases where one uses colposcopy alone, especially since among the old transformation zones and the transformation zones in the marginal regions of ectopies, carcinomas *in situ* are occasionally to be found. These do not display the corresponding colposcopical criteria. In our material, the malignancy index for the old transformation zone is 1.32 per cent (including dysplasias up to 3.1 per cent), as compared to 0.59 per cent for the true erosion (including dysplasias up to 2.14 per cent). I agree with Bret that cytology and colposcopy should be combined. The two methods should not compete with each other but supplement each other as proposed by Janisch in his discussion.

To Krüger: The error of colposcopy of more than 33 per cent can be explained by the systematic combination of three diagnostic methods. I do not think it is correct to use cytology only in colposcopically suspicious cases. By routine combination of the two methods a) the error will be smaller and b) the number of biopsies will be decreased. Here colposcopy offers the great advantage in that it allows one to take the cytological smear under guidance and to perform the biopsy on the correct site.

Colpomicroscopy of Carcinoma *In Situ*

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EKKEHARD KOFLER, HANNES KREMER

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(Director: Prof. Dr. T. Antoine)

ESSENTIALLY the picture is the same as with invasive carcinoma. The findings in carcinoma with the colpomicroscope are: characteristic changes like polymorphia, polychromasia, sometimes pathological mitoses, giant nuclei, loss of cellular borders and an increase in the number of atypical nuclei. Often a halo is found around the atypical nucleus. According to our experience this latter finding and the presence of numerous so-called "snake-cells" speaks for an already invasive carcinoma.

On the other hand one sees in histologically confirmed carcinoma *in situ* a lack of snake cells, a clearer cell picture; a crowding of nuclei in the field of view is not as pronounced as in invasive carcinoma and rarely marked inflammatory signs are present.

A safe distinction between carcinoma *in situ* and invasive carcinoma cannot be done by means of colpomicroscopy, as it cannot by means of cytology.

WOLFGANG WALZ

From the Department of Obstetrics and Gynecology, Kreiskrankenhaus, Heidenheim an der Brenz, Germany

A SAFE differentiation between an invasive carcinoma and a carcinoma *in situ* by colpomicroscope is not possible as only the cell stratum on the surface is observable. This stratum does not show specific characteristic signs of a carcinoma *in situ*, thus nothing definite can be said about a possible invasive growth. For this reason no therapy must be started without a preceding histological examination. However, we are trying to show certain characteristics appearing with carcinoma *in situ* based on our colpomicroscopical findings. Perhaps these experiences can give certain clues to the cytological diagnosis as we observe colpomicroscopically the same cells, which are found in the cytological smear: cells observed by the colpomicroscope in their original unit and surroundings.

Pertaining to the growth of carcinoma *in situ* we differentiate between two main types: 1) a coherently covered surface with or without formation of a needle-shaped growth; 2) an isolated, primarily needle-shaped growth within an ectopy or a true erosion.

Corresponding to the histological findings we can differentiate, colpomicroscopically, degrees of maturity of carcinoma *in situ*: a) predominantly polymorph, b) semimature, and c) mature partly cornified.

Type 1. This kind of growth being the most frequent, it presents no difficulties to a colpomicroscopical diagnosis. The border between normal squamous epithelium and the carcinoma is always a sharp one. Frequently at this border there is an area of cells with small nuclei. These cells stain intensely and usually possess no chromatin structure. They are rather uniform in shape and only rarely of abnormal size; polymorphism and polychromasia are not often

evident. They are situated regularly, cell boundaries being absent. Individually observed, such cells would not be regarded as malignant. We regard them as dyskaryotic cells (Fig. 1). Joining this area we find the true carcinoma tissue, recognizable by polymorphism and polychromasia of cells and their nuclei. In a case of histologically immature carcinoma the nuclei are predominantly small, intensely stained and situated close to each other. The more mature carcinomas *in situ* are recognizable by bigger nuclei of very irregular shape with clear chromatin structure and a plurality of nucleoli. The nuclei are not positioned as closely. The picture made by all types of these cells is an irregular interspersing within an onion-peel or vortex-like pattern. This is caused by connective papillae, which have penetrated the epithelium close to its surface. In the neighborhood of these papillae there frequently are spindle cells (Fig. 2). Sometimes there are mitoses on the surface. In some cases one can observe the proliferation of a carcinomatous epithelium into the duct of a cervical gland, if this duct leads to the surface. The border to the cervical canal is not accessible for observation as this border in most cases is situated directly at the os externum or slightly above. In general the surface of a carcinoma *in situ* is very clear and level, contrary to an invasive carcinoma.

Type 2. For this kind of growth the diagnosis by aid of colpomicroscope is somewhat difficult—the situation not being as clear. The difficulty is caused by the production of mucus of a possible ectopic os by an accompanying inflammation in case of a true erosion. The carcinomatous isles are situated within an ectopy or, most frequently, within a chronically inflammatory infiltrated tissue, the columnar epithelium usually having been destroyed. These carcinomatous units are recognizable by polymorphism and polychromasia of their nuclei, *i.e.*, similar to criteria as described under Type 1, with the sole exception that

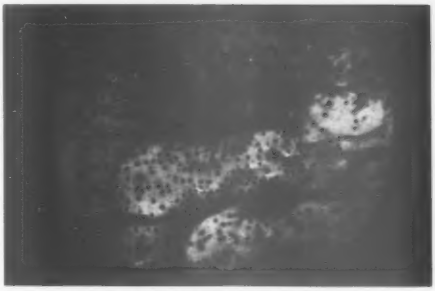

FIG. 1. Marginal area of a carcinoma *in situ*. Lower right: normal squamous epithelium. Upper left: area with dyskaryotic cells bordered by the real carcinoma *in situ*. This and the following pictures stained with toluidine-blue (180x).

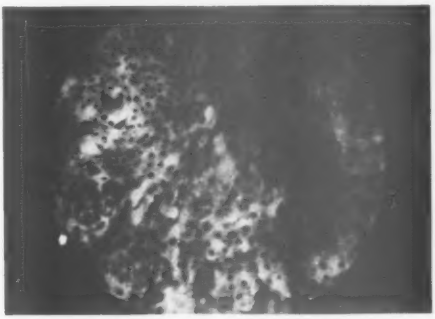

FIG. 2. Same diagnosis as Figure 1. Border between the dyskaryotic-cell-zone and the center. Giant cells and few spindle cells (180x).

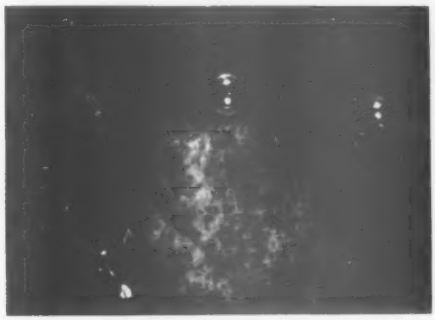

FIG. 3. Isle of carcinoma in the midst of an ectopy, upper part of picture. Disturbed positioning is apparent. Polymorphism and polychromasia of cells.

TABLE 1.

Sign	Inflammatory epithelium	Indirect metaplasia	Dysplasia "unquiet epithelium"	Carcinoma *in situ*	Invasive carcinoma
Border towards normal squamous epithelium	vague	sharp or transformation-zone in between	sharp	sharp	usually sharp, occasionally inflamed marginal area
Surface	not clear, but level	usually not clear, mostly level	clear, level	clear, mostly level	marginal area—clear, center area—not clear, not level
Connective papillae	few	many	most frequent	many	many
Polymorphism of cells and nuclei	little—moderate	little, some giant nuclei	moderate	strong	strong
Polychromasia	usually weak staining	little	little—moderate	strong	strong
Cell boundaries	usually apparent	always apparent	changing	lacking	lacking
Cellular debris	much	no	no	no	much in case of necrosis
Inflammatory cells	many	few	lacking	lacking	existing
Dyskaryotic cells	few, dispersed	no	few, dispersed	many, mostly at the border	moderate dispersed
Spindle cells	few	no	few	frequent	frequent
Mitoses	no	very rarely	no	frequent	frequent

the finding does not appear as clearly because of influences of the surrounding area (Fig. 3). In this case, too, one can frequently observe the proliferation of the epithelium into the cervical glands. The border towards the adjoining tissue usually is not well defined. Thus it is always important to consider the behavior of such an epithelium in regard to its immediate surroundings.

Difficulties in the differential diagnosis of a carcinoma *in situ* can exist in respect to an inflammatory epithelium, a dysplasia ("unquiet epithelium," nomenclature of Zürich school) and an indirect metaplasia. We have listed the most important symptoms of differential diagnosis, calling special attention to the characteristics peculiar to carcinoma *in situ* (Table 1). However, we would like to warn one not to overestimate the value of these characterisics in relation to an invasive carcinoma. In our method of surface observation there is only a small degree of differentiation, particularly in the case of a beginning carcinoma with intact surface.

In conclusion I would like to point out some important findings for the cytological diagnosis of carcinoma *in situ* from our experience. An increase of number of dyskaryotic cells, as found statistically by Wied,[5, 6] can also be proved colpomicroscopically. Most carcinomas *in situ*, particularly in the child-bearing age and if

there is no important accompanying inflammation, yield a "clear smear," *i.e.*, only a few leukocytes and lymphocytes. Other colpomicroscopical characteristics which might indicate a carcinoma *in situ* are not reproducible by cytological smears due to the fact that they appear only in the tissue as a whole.

Bibliography

1. Antoine, T. and V. Grünberger: Atlas d. Kolpomikroskopie. Stuttgart, Georg Thieme, 1956.
2. Walz, W.: Z. Geburtsh, 144: 117, 1955.
3. ——: Geburtsh. u. Frauenhk. 18: 243, 1958.
4. ——: Acta Cytologica. 2: 17, 1958.
5. Wied, G. L. and A. M. Dargan: Arch. Gynak. 189: 358, 1957.
6. Wied, G. L.: Am. J. Obst. & Gyn. 71: 793, 1956.

Discussion

Marco Marcov, Stara-Zagora, Bulgaria: Walz's paper on colpomicroscopy of carcinoma *in situ* is so lucid that there is indeed very little to discuss.

Doubtless colpomicroscopy has potential value in detection of early carcinoma, especially of the cervix. This method permits a direct visualization of such characteristics as anisokaryosis, hyperchromasia and anisocytosis in undisturbed sheets of superficial cells.

We are in full agreement with the author, that a differentiation between an invasive carcinoma and a carcinoma *in situ* by colpomicroscopy is impossible.

Regular examinations by colpomicroscopy give the same results as cytological smears.

I would like to ask one question: Is the determination of the degree of maturity of the cells more exact by colpomicroscopy than by cytological smears?

Closing Remarks

Tassilo Antoine, Kurt Brandl, Viktor Grünberger, Ekkehard Kofler and Hannes Kremer: As far as we are concerned the determination of the degree of maturity of the cells is equally exact in both early cancer detection methods, colpomicroscopy and cytology.

Wolfgang Walz: The determination of the degree of maturation of a carcinoma *in situ* is, in most cases, safer by means of the colpomicroscope than by means of cytology, since colpomicroscopically the lesion can be visualized in its entirety. The histologically mature carcinoma *in situ* which is tending toward cornification can be recognized colpomicroscopically by its macronucleated cells and the relatively large distance between the nuclei. In between, cornifying areas may be found. The less mature a carcinoma *in situ* is, the denser the nuclei are and the more polymorphism can be seen. Mostly the nuclei are richer in chromatin and therefore stain darker. In general, the nuclear size is smaller in the more immature forms as compared to the mature lesions.

Schiller Test on Carcinoma *In Situ*

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CYTOLOGY and colposcopy have a universally accepted value in the recognition of early cancer. The stratified squamous epithelium of the portio and the vagina is rich in glycogen, especially in women of childbearing age. Cancerous epithelium is, as a rule, glycolytic. Schiller took advantage of this fact and introduced the iodine test in the early diagnosis of carcinoma of the cervix. The test consists of staining the

cervix or the vagina thoroughly with Lugol or with a solution of one part iodine and two parts potassium iodine to 300 parts water. Also, a stronger solution is satisfactory. For many years I have been using iodine alcohol. Excessive mucous secretions should be gently removed prior to the staining of the mucosa with the solution.

The squamous epithelium rich in glycogen, takes a homogenous, deep-brown or blue-brown stain, while glycolytic areas become only light-brown. Often there is a sharp contrast between the areas. From the glycolytic areas the physician will take several small pieces with a punch. He can easily perform this procedure in his private office without anesthesia. The pathologist will often be unable to give an ultimate diagnosis on the small biopsies taken, but he may easily throw suspicion upon the presence of an early carcinoma. In this respect, the Schiller tinctorial test has proved to be of value in the early detection of a carcinoma *in situ*. It should, however, be emphasized that the iodine test is not specific for carcinoma. Leukoplakia, erosions of different kinds and an ulceration of the mucosa also remain unstained.

In addition, the iodine test is of value in deciding upon the size of the area which should be removed in cases of carcinoma *in situ* selected for conization.

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THE EFFECTIVENESS of the Schiller test for the diagnosis of carcinoma *in situ* should be considered from two different viewpoints — as a complement to both the macroscopic and the colposcopic examination.

As a complement to the macroscopic examination the method undoubtedly has great advantages. The presence of iodine negative areas in the cervix cannot be accepted as an absolute indication for the atypical condition of an epithelium. We must bear in mind that although there may exist other modifications of the mucosa (inflammatory conditions, ectopia, processes of regeneration and epithelia with a decreased glycogen content) which do not stain with iodine, iodine negative zones are due to abnormal epithelia in a high percentage of the cases. For these reasons I believe that for the gynecologist, who is against colposcopy as an exploratory method, and for the general practitioner, who does not have a colposcope available, the Schiller test is a valuable help for discovering these abnormal epithelia and for directing the course of the biopsy.

As a complement to the colposcopic examination the advantages of the method are somewhat reduced, since generally the observation of the cervix with acetic acid and silver nitrate permits the recognition of abnormal epithelia. Nevertheless, we perform the Schiller test systematically prior to the second colposcopic examination. Sometimes it discovers small iodine negative areas which might have remained overlooked, and it defines the suspicious zone and directs and circumscribes the biopsy.

Having discussed both viewpoints, we also would like to comment on the expediency in other circumstances, for instance when effected prior to an extensive biopsy (conization or amputation) when a biopsy has to be performed in a cervix that already colposcopically revealed suspicious epithelia. Then the method will help define the extension of the area that will have to

be extirpated. In the cases where a simple biopsy discovered a carcinoma *in situ*, an extensive biopsy and the corresponding series of sections will reveal if somewhere the carcinoma has reached the invasive stage. Here the Schiller test will be a valuable help to determine the limits of the excision and show the way to a conization or tell us if a wider area has to be amputated. On certain occasions one can even observe fornices of the atypical epithelium.

I definitely consider the Schiller test a very valuable method as a complement to the macroscopic and colposcopic examination of the cervix.

ALFRED WACEK, JACQUES W. JENNY

From the Department of Obstetrics and Gynecology, University of Zürich, Zürich, Switzerland (Director: Prof. Dr. E. Held)

IN 1928, W. Schiller introduced the iodine test into the early diagnosis of cervical cancer.

The normal squamous epithelium of the vagina and the outer surface of the cervix contains glycogen. With a Lugol's solution of 1-2 per cent iodine content, this glycogen gives a deep brown stain. Any deviation of the normal structure of the epithelium is connected with a loss of the glycogen formation and subsequently of the iodine stain.

Already in his first publication, Schiller pointed to the fact that this test is not at all specific for carcinoma or its early stages. Any change of the epithelial structure, where the glycogen content is lacking or only poorly developed, gives a negative or poor coloration. This is the case in such benign changes as ectopy of the columnar epithelium of the cervix, inflammatory lesions or atrophy of the squamous epithelium.

Therefore the Schiller test can only be used as a screening method. If the entire surface of the cervix shows a brown stain (Schiller test negative), premalignant or malignant changes of the visible epithelium can be excluded. All iodine-negative areas (Schiller test positive) have to be further evaluated, either by biopsy, colposcopy or cytology.

Although the Schiller test has become universally accepted as a very helpful aid in detecting changes of the cervical epithelium—a gynecological examination today is considered incomplete without its application—further studies have shown that it also has its failures. The epithelial changes might take place within the cervical canal and therefore be hidden to direct inspection. The surface of the cervix can be completely unsuspicious and iodine-positive and yet a carcinoma *in situ* might develop intracervically. Only cytology might detect these cases or they might be casual findings in operative preparations. There exists a wide variation of opinion about the frequency of these intracervical lesions. Some authors report ten or more per cent, *e.g.*, Zinser 10-30 per cent, whereas Held found only 2.5 per cent in his material, and Limburg, 2.9 per cent.

Of 104 cases of carcinoma *in situ*, admitted to the University Hospital for Women, Zürich, within the last years, 103 had a "positive" Schiller test, *i.e.*, showed an iodine-negative area of varying extent. Only one case had a completely normal appearing cervix with iodine-positive staining. In 54 cases of the 103 the iodine-negative area was limited to the inner third around the cervical os, in 43 cases it extended over two thirds, in two cases over the entire cervix and in four cases into the vaginal vault. There was no specific shape

or appearance of these areas which would have led to the diagnosis prior to further evaluation (cytology, colposcopy, biopsy).

The discrepancy in the frequency of observation of the intracervical carcinoma *in situ* might possibly be based on the method of inspection of the cervix. At the University Hospital for Women, Zürich, a bivalve speculum is used routinely for inspection of the cervix. By this method the anterior and posterior vaginal vaults are widely spread and the cervical os opens slightly. It is possible to inspect at least the lowest fourth of the cervical canal. Changes within this area are therefore noted, including changes in the Schiller test.

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THE major interest of the Schiller test is to visualize abnormal epithelium (benign or suspicious dysplasias; early, non-ulcerated carcinoma) on the normal looking cervix.

Differences of opinion on the significance of the Schiller test are due to a confusion between its relative value in the obvious carcinoma and its real value in lesions still *in situ*.

Indeed, for detecting the clinical and especially the ulcerated cancers, the test loses its value, revealing nothing more than the speculum examination. However, it does detect the presence and extent of any atypical areas surrounding the invasive carcinoma.

Moricard's research on the glycogen content of the normal and abnormal cervical epithelium leads to the following conclusions:

1. The absence of glycogen is not a specific criterion of malignant change, but it is a usual characteristic.

2. The sudden disappearance of glycogen in carcinoma *in situ* is practically without exception.

3. The persistence of an abundant content of glycogen in carcinomas is exceptional (2.7 per cent in invasive carcinomas). One would almost always be dealing with clinically obvious invasive carcinomas. The topography of such a content is abnormal.

Therefore, we may emphasize that whenever the content of glycogen is normal, any *in situ* is excluded.

Interpretation and Results

The major inconvenience of the Lugol test is that it does not define the nature of the lesions precisely.

Therefore, the classical description of the Lugol-negative or abnormal areas is imprecise and implies a large number of useless biopsies.

A methodical interpretation, based on the systematic analysis of the exact color and especially the borders of the glycogen-negative area permits a classification into three groups of precise meaning:

1. Red Areas
   - —an absence of epithelium: erosion and ulceration;
   - —the presence of columnar epithelium; ectopy or ectropion;
   - —the presence of a thin squamous epithelium: atrophy, hypoplasia, epidermization area with a few layers, neoplasia in the process of ulceration, etc.

The Schiller test has no significance in the study of these zones. Lugol does not change them. These areas are to be studied by colposcopy which does not discover more suspicious ones, but does at once eliminate the majority of them as benign.

2. White Areas

They are related to an abnormal squamous epithelium. The quality of the negativity (which can be partial or total, homogenous or irregular) and the aspect of the contours (which can be sharp or faint) —such a distinction seems to be essential— permits a valuable differentiation in benign and suspicious zones.

A. *Iodine negative areas with faint borders*

They correspond mainly to 1) epidermization areas recovering their glycogen content, 2) inflammations, and 3) hormonal dystrophies or dystrophies due to abnormal receptivity. These are essentially benign.

B. *Iodine negative areas with sharp borders*

They are of major interest in carcinoma *in situ*, which usually presents this aspect. They are not characteristic and may be indistinctly related to a regular dysplasia, an irregular dysplasia, a carcinoma *in situ*, or even to an invasive carcinoma. Only histology can determine their true nature.

Undoubtedly, colposcopy definitely interprets certain complex aspects, namely, the sharpness of the borders.

The differential tests with estrogens (Palmer) and antibiotics allow the determination in some cases of the hormonal or inflammatory origin of a lesion.

Statistical Correlations Between Schiller's Test and Biopsies

Nineteen carcinomas *in situ* showed 17 iodine negative areas with sharp borders, one red area shown in a case of endocervical carcinoma *in situ* detected by ectocervical smears, to one iodine negative area with faint borders, histologically unsuspected.

These data emphasize:

—the suspicious character of iodine negative areas with sharp borders (index of malignancy 10.9 per cent);

—the general benign character of iodine negative areas with faint borders (no suspicious lesion);

—valuelessness of the test for detection of endocervical lesions.

Three carcinomas *in situ* were detected on iodine negative areas with sharp borders without any colposcopical special pattern.

Conclusion: The Schiller test cannot be used to recognize whether a lesion is or is not neoplastic, but it does eliminate at once any suspicious ectocervical lesion when the cervix stains normally after Lugol. It contributes not only to the early detection of cervical carcinoma, but also and above all to its prophylaxis.

In this field its value is limited by its failure in detecting endocervical lesions. The importance of this is a function of the frequency of primarily endocervical malignant lesions. However, this does constitute a serious argument against its exclusive use for carcinoma detection.

It permits the localization of biopsies and treatments. It is an indispensable complement of colposcopy.

Taking into account its limits and its lack of specificity, the Schiller test, because it is easy and fast, would be a systematic step in every complete gynecological examination.

Bibliography

1. Palmer, R.: Revue française de Gynecologie, 1947, p. 85.
2. Funck-Brentano, P., R. Moricard, R. Palmer and J. DeBrux: (Congres de gynecologie Alger May 1952) Bull. Fed. Soc. Gyn. Obst. 4: 1, 1952.
3. Moricard, R.: Gyn. et Obst. 51: 1, 1954.
4. ——: Bull. Fed. Soc. Gyn. Obst. 6: 5, 1954.

Discussion

Michael J. Jordan, Genevieve M. Bader and Emerson Day, New York, New York, U.S.A.: For the past nine years we have been using tincture of iodine, strong N.F. (7% iodine, 5% potassium iodide, 83% alcohol) in our cervix study. While various older sources in the literature state that the aqueous solution is preferable, no reasons are given for not using an alcoholic solution. More

recent investigations, namely our own, Ferguson's, and Kottmeier's, have shown that the strong tincture is preferable for demarcating areas of glycogen depletion. Several reasons led to our adoption of this solution over others. First, we tried the various aqueous solutions before using the alcoholic tincture. Aqueous solutions proved unsatisfactory chiefly because of instability. They are relatively inert and useless unless freshly made up. The strong tincture gave the best results from the standpoint of demarcation of the lesion. This has been proved by our results in photographing the cervix.

The atypical lesions of the cervix under study do not take up the iodine stain because of the absence of glycogen. We are aware of the fragility of the lesion called carcinoma *in situ*, and therefore the iodine is applied gently with a cotton-tipped applicator. A minimal amount of iodine (less than ¼ cc.) is used, in contrast to the 20-30 cc. with which Schiller bathed the cervix and vagina. It has been noted that on reintroduction of the speculum after completion of the bimanual examination using K-Y jelly, the iodine stain has almost completely disappeared.

We have been using the same staining technic for over nine years, and at no time have we been informed that repeat smears (some repeated within one week after staining) have shown a cautery effect due to the use of iodine. Nor has there been any effect shown on repeat biopsies after the solution has been used frequently.

We have searched the literature very carefully and except for a statement by Limburg (who states that any iodine solution may have a "cautery effect" on the superficial cervical epithelium) we have no other data which would either support or nullify the use of 7 per cent alcoholic iodine.

Ernst-Helmuth Krüger, Halle a.d. Saale, Germany: I consider the iodine test generally superfluous and do not think that it is a necessary extension of colposcopy. One who does not know how to use the colposcope should use iodine, but one who knows how to use the colposcope may skip it without diminishing the advantages. Iodine negative and colposcopically negative areas do not need, in our opinion, histological clarification. In the search for the so-called carcinoma *in situ* the iodine test has not been of great help to us. The test is best in determining the extent of the biopsy to be performed and also in determining whether or not all atypical tissue has been removed. Contrary to Kottmeier, we merely use a solution of iodine: potassium-iodide: water in the relationship of 1:2: 300 or 1:2:500, respectively.

Warren R. Lang, Philadelphia, Pennsylvania, U.S.A.: As has been mentioned by the above authors, the Schiller test shows an absence of brown staining when glycogen is scanty or lacking. This latter situation is found when (a) squamous epithelium is atrophic, inflamed, regenerating, atypical, preinvasive carcinoma or invasive carcinoma, (b) there is columnar epithelium, (c) there is absent epithelium.

Our experience would agree with Vasquez-Ferro that the method adds little to colposcopy. The Schiller technic can be utilized as a screening method although, because of its nonspecific nature, there are many "false positives." The experience of Wacek and Jenny in screening for carcinoma *in situ* and finding lack of staining in 103 of 104 cases *in situ* carcinoma is significant. This lends some support to those, now apparently in the minority, who believe that *in situ* lesions often originate on the portio.

As an added word of caution; the use of the Schiller test will change the level of protein-bound iodine.

Marco Marcov, Stara-Zagora, Bulgaria: In my opinion the Schiller test should be used at every gynecological examination, because of its simplicity. When applied in the broad gynecological practice, this test, even when used by a less experienced physician, will at least show where the biopsy can be most properly done.

Hans-Klaus Zinser, Cologne, Germany: It has been indicated already by the main speakers that there is no such thing as specificity of the Schiller test for recognition of carcinoma *in situ*. I would like to mention the work by Andersen and coworkers (Acta Obst. Scand. 23: 26, 1942) who have found among 106 iodine negative areas only one true carcinoma and six "precancerous" changes. If one would subject all the iodine negative cervices to histological examination the biopsy rate would increase beyond all permissible limits and not even then would all carcinomas *in situ* be discovered. Also, in the presence of colposcopically suspicious findings, the iodine test is, as a rule, only an additional confirmation, without enabling us to safely exclude atypical epithelium if the test is negative. In my opinion the iodine method has lost much of its importance since the introduction of cytology. To me it seems more useful to clarify the colposcopically suspicious or colposcopically uncertain findings by means of the cytological smear. For the clinician who obtains his findings only macroscopically the iodine test may represent a crude preselective method in order to achieve some refinement of his diagnosis, but no optimal result can be expected for the early diagnosis of malignancy.

Closing Remarks

Hans-Ludvig Kottmeier: As mentioned in my main paper, the iodine test has a limited value in the early detection of carcinoma *in situ*. This is, however, valid for a gynecologist. In my opinion, colposcopy should only be performed by trained gynecologists and should not be used by general practitioners. The use of the Schiller iodine test will improve the possibilities of detecting early carcinoma *in situ* by general practioners.

I do agree with Jordan *et al.* that the stronger iodine tincture does not have any significant "cautery effect."

Enrique Vasquez Ferro: We prefer, as do Jordan, Genevieve Bader and Day, strong solutions for the Schiller test, but we did not find important differences between tincture of iodine or Lugol's solution. We agree with Krüger and Lang that the advantages of the Schiller test are limited in colposcopical examinations, but the test becomes of importance in routine gynecological examinations. It is gratifying to have read Zinser's statements.

Alfred Wacek: To Jordan *et al.*: We have had no personal experience with an alcoholic solution of iodine. We have always used the original aqueous Lugol's solution recommended by Schiller. We therefore cannot comment on this but would like to quote Navratil who states ". . . that the alcoholic solution . . . shows the difference in staining only for a few seconds. The necrosis of the superficial cells due to the alcohol leads to an absorption of the solution which makes it impossible to differentiate between normal and carcinomatous epithelium."[1]

To Krüger: We believe that the application of the Schiller test is a necessary element of colposcopy. There are the so-called uncharacteristic iodine negative areas with sharp borders ("uncharakteristischer scharfandig jodnegativer Bezirk") which would be missed on routine colposcopy without application of iodine. They were found in 46.5 per cent of 3,243 unselected patients of our colposcopy station and the biopsy revealed a carcinoma *in situ* in about 1 per cent (cf. Wyss, to be published).

To Zinser: We agree that for the gynecologist performing routine colposcopy and cytology the iodine test may be unnecessary. Yet we would not like to omit it from our colposcopic examinations since there are many colposcopically negative or uncertain findings which can be clarified by the iodine test. We refer especially to the uncharacteristic iodine negative areas with sharp borders (see our remark to the article of Krüger). These areas need to be evaluated by biopsy unless they are small and within the iodine positive area far from the cervical os or only with a narrow connection with the latter not wider than 3 mm. in diameter. In these cases we have abandoned a routine biopsy since our experience has shown that these areas almost never contain atypical epithelium (Wyss).

For the general practitioner without training in colposcopy and not doing a routine cytological smear (a goal which we here in Europe still are far from), the iodine test is a helpful, simple and necessary adjunct in evaluating the cervix. It allows a crude selection of the material. All cases with iodine negative areas should be further evaluated either by cytology, if the facilities of a cytological laboratory exist (as it is the case in our metropolitan area) or referred to a gynecologist for colposcopy. We do not recommend a routine biopsy in these cases. We agree with Zinser that the biopsy rate would then exceed the possibilities of any histology laboratory.

We are fully aware of the fact that with this policy some truly intracervical lesions are missed, but as shown by our figures on the carcinoma *in situ* they are rare.[2] In the field of the general practioner we have to find a compromise between the ideal we are striving for and our present facilities.

Bibliography

1. Navratil: Biologie und Pathologie des Weibes, **IV:** 668, 1955.
2. Held: Archiv. für Gynäkologie. **188:** 376, 1957.

Henriette Wenner-Mangen: Since the aqueous solution (1 part iodine, 2 parts potassium iodide, 300 parts water) has given us satisfactory results we did not employ the strong alcohqlic solution recommended by Kottmeier. As opposed to Jordan, Genevieve Bader and Day, we observed an extensive whitening of the cervix. The histological examination has never been disturbed by the previous application of Lugol's solution. As a rule the vaginal and ectocervical smears are taken prior to the Schiller test.

I should like to reply to Krüger, that the problem of the sharply outlined iodine negative areas, without particular colposcopical findings, poses different problems from the theoretical as well as from the practical points of view. Alerted by the Schiller test one may find at subsequent colposcopy areas, slightly different from normal; a little more whitish, more dull or more shiny. These patterns can easily escape detection on routine examination, even when performed by an experienced colposcopist. On the other hand a histologically abnormal finding is not necessarily brought to attention by colposcopy when the lesion does not represent a topographical change and particularly when the parallelism between surface and basal layers is preserved. On the contrary, topographically, these zones may well correspond to a carcinoma *in situ*.

From the viewpoint of exclusive detection of carcinoma *in situ* I realize, together with Zinser, that the Schiller test. even if interpreted correctly, entails a great number of useless biopsies. Their number could perhaps be reduced by systematically combining colposcopy with cytology. In addition, in those cases with positive or suspicious smears we perform biopsies on all cases which show the presence of dysplastic and dyskaryotic cells. Our goal is to discern the dysplasias and to remove them for prophylactic purposes.

III. Exfoliative Cytology and Experimental Cytology of Carcinoma *In Situ*

Cytomorphology of Carcinoma *In Situ*

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THE CYTOLOGICAL picture of the carcinoma *in situ* is monomorphous, constituted by immature cells of basal type, with here and there a very few mature elements, some of which may have the morphology of a fiber cell. These elements are sometimes isolated, but typically are found in small groups or patches, or disposed in bands; the cellular contours are always perfectly limited, and never form strips of tissue in which the cells are joined.

The characteristic aspect of the carcinoma *in situ*—rather rarely realized, moreover—is that of a trail of lined-up cells, all cyanophilic except for one or two whose very chromatic nucleus is surrounded by an eosinophilic or orange-tinged cytoplasm (Fig. 1, 2).

a. The nuclear aspects are variable. The nucleus is immature, approximately round and situated in the exact center of the cell; its striking features are its volume, which inverses the normal nuclear-cytoplasmic ratio, and the rather coarse structure of its chromatin. Studied in greater detail, it is found to have a thickened nuclear membrane, usually regular but in some cases notched, and the chromatin appears disposed in thick clumps packed together without visible nucleoli (Fig. 3).

b. At the level of the elements in process of maturation or already mature, which form the very superficial layers of the carcinoma *in situ*, the nuclei are much more mature, forming an intense black mass of irregular contours, festooned or angular, whose chromatin structure is no longer discernible. They are roughly round, or slightly elongated parallel to the long axis of the cell, which becomes fibroid. The cytoplasm may be either cyanophilic or eosinophilic. Mitoses are few in number, and inflammatory elements are nearly absent (Fig. 4).

In summary, the existence of a carcinoma *in situ* may be recognized by the presence of small basal cells, for the most part immature, and whose nuclear anomalies, *a priori* seemingly slight, require a close examination on higher magnification.

However, as thus described, there are two benign lesions with which the carcinoma *in situ* may be confused, *e.g.*, (1) the active undifferentiated metaplasia (hyperplasia of the reserve cells), and (2) the irregular dysplasia.

In the carcinoma *in situ*, the nuclear anomalies correspond very nearly to the ideal criteria established for the malignant cell: increase of the nucleo-plasmic ratio, thickening of the nuclear membrane, and density of the chromatin, distributed in irregular clumps.

In the active undifferentiated metaplasia, all these aspects are found, but to a much lesser degree, as if just beginning; the nucleoplasmic ratio tends to be reversed, the nuclear membrane is accentuated, the chromatin is grainy—nuances only, sometimes very difficult to perceive, that distinguish this lesion from a carcinoma *in situ* that regress are in reality active metaplasias of this type, whose aspect is particularly deceptive both on the smear and on the biopsy, but especially on the latter, since between these two lesions innumerable gradations may exist.

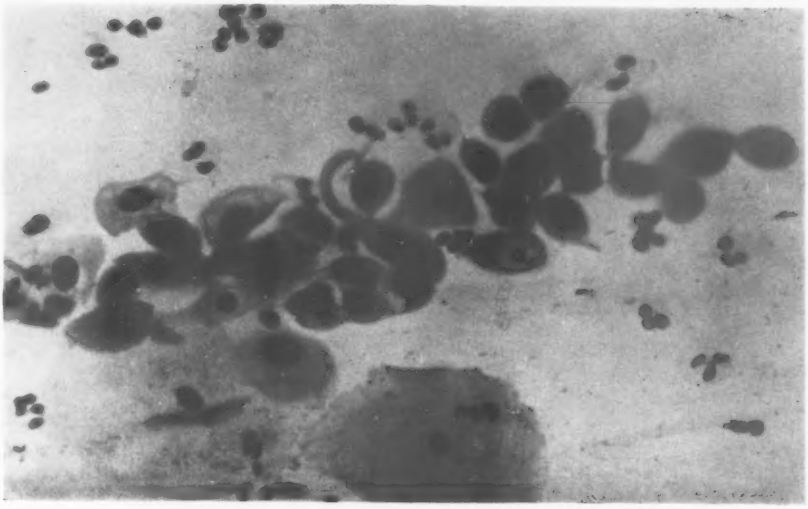

FIG. 1. Cells from a carcinoma *in situ* with hyperchromasia.

Nevertheless, two elements of differential diagnosis should be sought: in the carcinoma *in situ,* mature elements with dysplastic nuclei and precociously cornified cytoplasm, disposed in completely isolated groups or trails; in the metaplasia the elements are contiguous or agglutinated, and the cytoplasmic limits are indistinct.

In the case of the irregular dysplasias, the differential diagnosis is relatively easier. They present the same elements as in the carcinoma *in situ—i.e.,* immature cells with

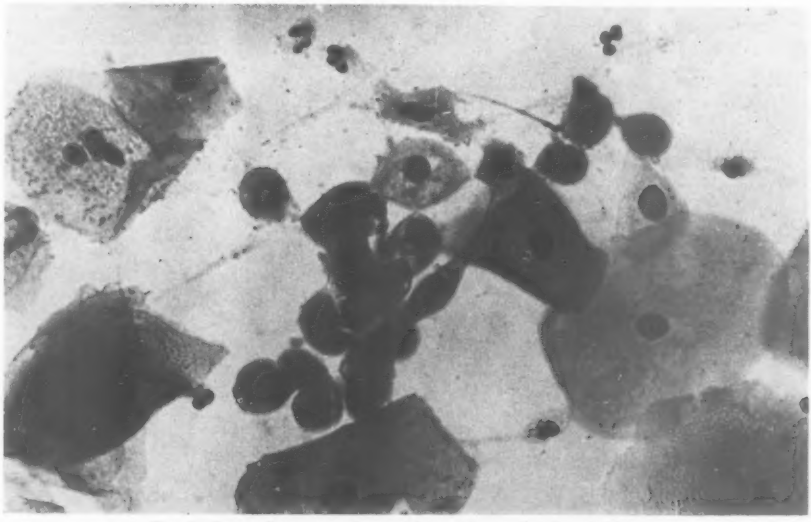

FIG. 2. Smear from a patient with carcinoma *in situ* which was histologically confirmed on the surgical specimen.

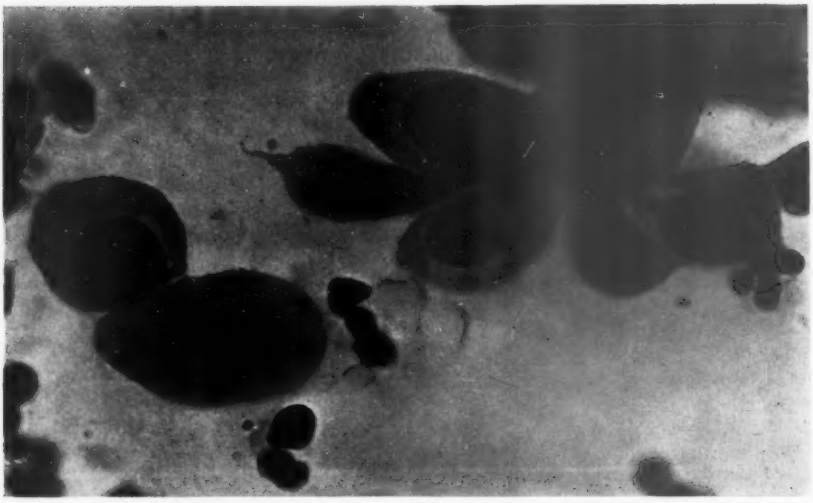

FIG. 3. Cells from a carcinoma *in situ*. Note the hyperchromasia unequally divided granules, thickening of the nuclear membrane and the regular outlines of the nuclei.

abnormal chromatin, and rounded or fibroid cells with dysplastic nuclei and cyanophilic or eosinophilic cytoplasm, but in inversed proportions:

1. the dysplasic cells are decidedly more numerous than the immature cells;
2. the eosinophilia is much more marked than the cyanophilia; and
3. the fiber-cells are in greater number.

As to the differential diagnosis with invasive carcinoma, this will be taken up under another topic.

FIG. 4. Cells from a carcinoma *in situ* in process of maturation. The nuclei are loaded with chromatin, distributed in irregular and unequal granules.

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IN order to determine the cytomorphology of carcinoma *in situ* in contrast to that of invasive carcinoma, differential counts were done on the type of malignant cell present in 105 cases of carcinoma *in situ* and 187 cases of invasive squamous carcinoma.

The percentage of three types of malignant cells has been determined. First is the undifferentiated malignant cell with marked chromatin irregularity, but with no cytoplasm or only tags of cytoplasm. No cell wall is present (see Fig. 1). Second, the third type differentiated squamous cancer cell. These are malignant cells with a definite cell wall, being oval or round in shape, and having less cytoplasm than the maximum diameter of the nucleus (see Fig. 2). Third, the differentiated fiber squamous carcinoma cell which has been discussed in a previous symposium and its characteristics defined.

A marked difference was seen in the percentage of undifferentiated cancer cells, as can be seen in Figure 3, where the invasive group is compared to the *in situ* group. Of the 187 cases of invasive squamous carcinoma only seven or 4 per cent had less than 25 per cent undifferentiated cancer in the vaginal smear. On the other hand the cells desquamating from the *in situ* lesions were more differentiated since 61 of the 105 cases or 57 per cent showed less than 25 per cent undifferentiated malignant cells. There were 30 cases in the *in situ* group with no undifferentiated cancer cells. In contrast, not a single case was encountered in the invasive group that did not have undifferentiated cancer cells.

If one considers the third type differentiated squamous cancer cell, it was found in every case of invasive cancer except three, supporting the thesis that this cell is a true malignant cell. As might be expected since differentiation was more pronounced in the *in situ* group, every case had these particular cells and they were much more frequent than in the invasive group. In the *in situ* carcinomas 72 or 69 per cent had more than 50 per cent of the malignant cell population classified as third type differentiated squamous cancer cells. This is in rather striking contrast to the invasive group where only 26 cases or 14 per cent showed this much differentiation. In the *in situ* cases there were 30 in which the third type differentiated cell was the only malignant cell encountered. No such

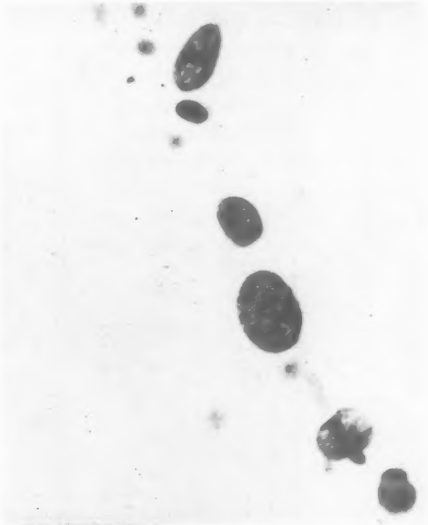

FIG. 1. Undifferentiated malignant cells with marked chromatin irregularity, but with no cytoplasm or only tags of cytoplasm. No cell wall is present.

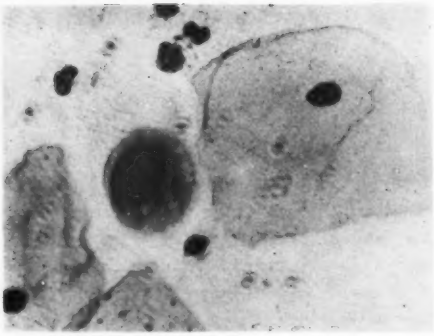

FIG. 2. Malignant cell with a definite cell wall, being oval or round in shape, and having less cytoplasm than the maximum diameter of the nucleus.

case was found in the 187 cases of invasive squamous carcinoma.

The smears from patients with invasive carcinoma differed in the presence of malignant fiber cells from the smears of patients with carcinoma *in situ*. Sixty-two per cent of the invasive lesions exhibited fiber cells in the vaginal smear. In the *in situ* group fiber cells were found in only three cases.

In summary, the morphological characteristics of carcinoma *in situ* as seen in desquamated cells is as follows. The picture is one of increased differentiation in contrast to the picture of invasive carcinoma. I consider the third type differentiated squamous cancer cell the most differentiated. In size and shape it may be identical to a benign basal cell except for its malignant nucleus. These cells are the characteristic ones of carcinoma *in situ*, occurring in every case and often being the only type of malignant cell encountered. Fiber cells are rare in carcinoma *in situ* in contrast to invasive carcinoma where they are a common finding. Finally, the undifferentiated cancer cell is much less common in carcinoma *in situ* than in invasive carcinoma. The entire cytologic picture indicates desquamation from a lesion which is more highly differentiated than that of invasive squamous carcinoma.

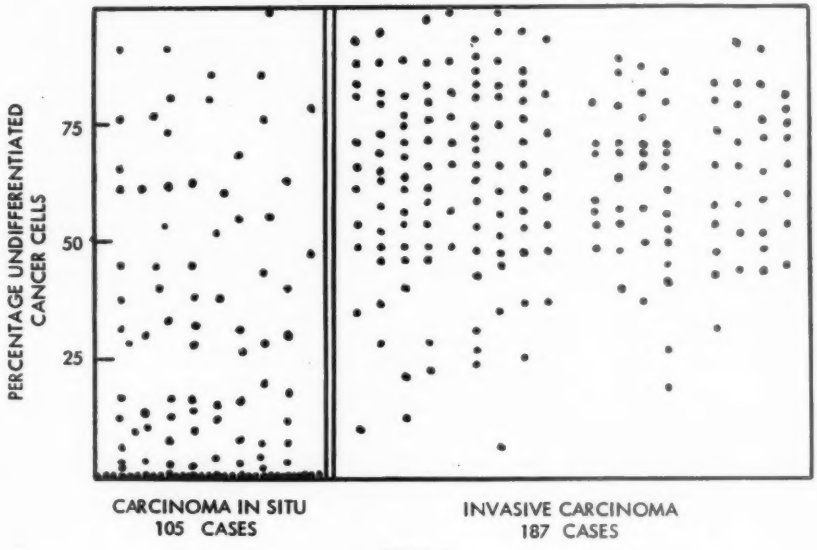

FIGURE 3

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MUCH has been written on this subject and the problem was discussed extensively at the First International Cancer Cytology Congress.[1] The most important fact to be gleaned from the literature is that the cytological pattern of carcinoma *in situ* must contain cells which by all present criteria can be considered malignant cells. This writer does not share the opinion of some authors that exfoliated malignant cells in lesions of carcinoma *in situ* can be effectively differentiated from malignant cells found in smears obtained from invasive carcinoma. Based upon my clinical experience as well as upon my experimental investigations, I feel that the malignant cells present in either lesion cannot be differentiated from those of the other and that any difference in cell pattern rests upon rather nonspecific criteria such as the presence of hemorrhage, infection, etc.[2]

It has been repeatedly emphasized that the principal malignant criteria rest upon changes in the nucleus and not in the cytoplasm. Enlargement of the nuclear mass with consequent changes in nuclear-cytoplasmic ratio is perhaps the most important single malignant characteristic. To this may be added hyperchromasia and the presence of multiple large or bizarre nucleoli. Giant-sized or multiploid nuclei found at random in smears of carcinoma *in situ* are less characteristic for this type of lesion than small tetraploid cells. All smears from carcinoma *in situ* should be classified at least as Class III, and more often as Class IV or V, by those following Papanicolaou's classification. The difference between the latter two classes rests mainly upon the percentage of those cells appearing in the smear, and this can often be correlated with the extent of the lesion. The appearance of the malignant nucleolus has been stressed as early as Quensel,[3] and confirmed by MacCarty,[4] von Haam[5] and others, but its absence does not exclude malignancy.

In addition to the appearance of malignant cells, the cell pattern of carcinoma *in situ* is characterized by a more or less pronounced dysplasia. It is very rare indeed that one finds malignant cells of carcinoma *in situ* in an otherwise perfectly normal vaginal smear. The dysplastic changes involve all cell layers and one always finds dyskaryotic basal cells and parabasal cells with increased white and red blood cells. The bacterial flora is not characteristic, and we certainly disagree with those who maintain that trichomoniasis is more frequently found in association with carcinoma *in situ*. Because of the fact that carcinoma *in situ* is always a lesion of the surface epithelium, we have found it easier to recognize in the vaginal smear than invasive carcinoma in which the cervical surface is often necrotic and does not contain many preserved cancer cells. The possibility that carcinoma of the cervix may develop without going through a stage of carcinoma *in situ* seems contraindicated by our experimental experience. Unless the cancer is too extensive, careful search will always show areas of carcinoma *in situ* beside the invasive foci. Similar observations have also been made in the experimental production of this lesion.[6]

Bibliography

1. Transactions, First International Cancer Cytology Congress, Chicago, Ill., Oct. 8-11, 1956.
2. Smolka, H. and H.-J. Soost: Grundriss und Atlas der Gynäkologischen Cytodiagnostik. Stuttgart, 1956, Georg Thieme.
3. Quensel, U.: Untersuchungen ueber die Morphologie des Harnsedimentes bei Krankheiten der Niere und der Harnwege. Stockholm, 1918, Nordiska Bokhandeln.
4. MacCarty, W. C.: Arch. Clin. Cancer Research. 1: 11, 1925.
5. Von Haam, E. and H. G. Alexander: Am. J. Clin. Path. 6: 394, 1936.
6. Von Haam, E. and D. G. Scarpelli: Cancer Research. 15: 449, 1955.

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IN order to discuss cytology of carcinoma *in situ*, one must realize that since the histological pattern is so variable, that the cytological pattern must also, therefore, be equally variable. In this present discussion, five different such patterns will be demonstrated by photomicrographs. This is not to imply that these are all of the vari-

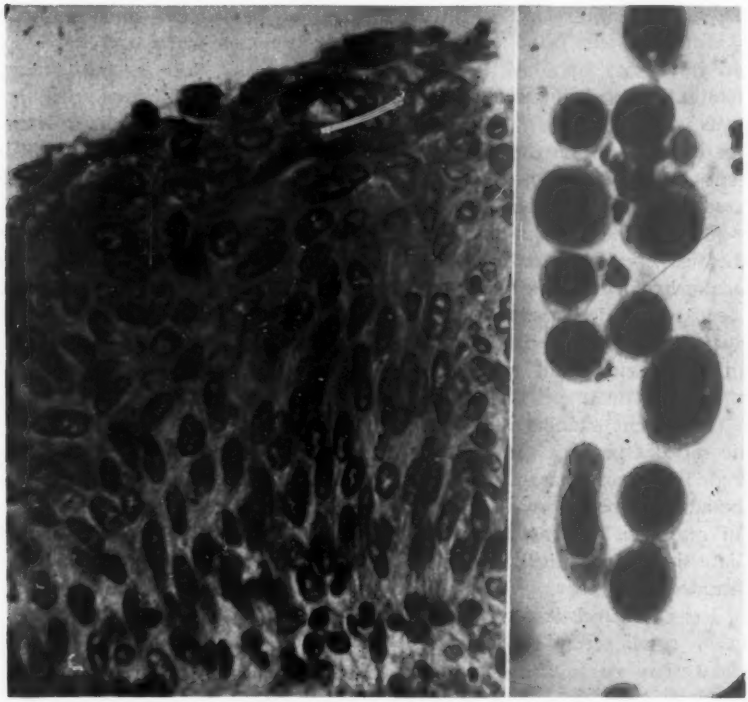


FIG. 1 illustrates the most important single feature of the positive exfoliated cells in this first case, namely, the lack of marked variation in size and shape of the nuclei. When the overwhelming majority of the positive exfoliated cells are of the basal cell type, one can be rather certain that histologically the lesion will be a carcinoma *in situ*. These cells are the most common type exfoliated by an *in situ* carcinoma. The nuclei of the tissue cells also exhibit relatively little variation in size and shape, they are hyperchromatic and the cells show marked crowding. This crowding effect correlates with the narrow rim of cytoplasm seen cytologically. On examining the tissue, it is best to concentrate on the surface cells since the exfoliated cells are more like the superficial cells, immediately before shedding.

ants. For example, in the cytological endocervical dyskaryosis pattern, we have been unable to convince ourselves of a distinctive tissue pattern which correlates. This should not, however, deter one from continually studying the tissue sections very carefully, with the eye of a cytologist, after the diagnostic criteria of carcinoma *in situ* has been adequately met by one's own standards.

The following cases were taken from a study group of two hundred patients who were biopsied because of abnormal cytological smears. In our laboratory we not only try to predict whether the lesion to be biopsied will be atypical hyperplasia, carcinoma *in situ* or invasive carcinoma of the cervix, but also try, for our own edification only, to see whether we can predict the type of atypia or *in situ* lesion present.

The photographs contain a cytological representative area on the right at a 430 magnification while the tissue section on the left is at a 350 magnification.

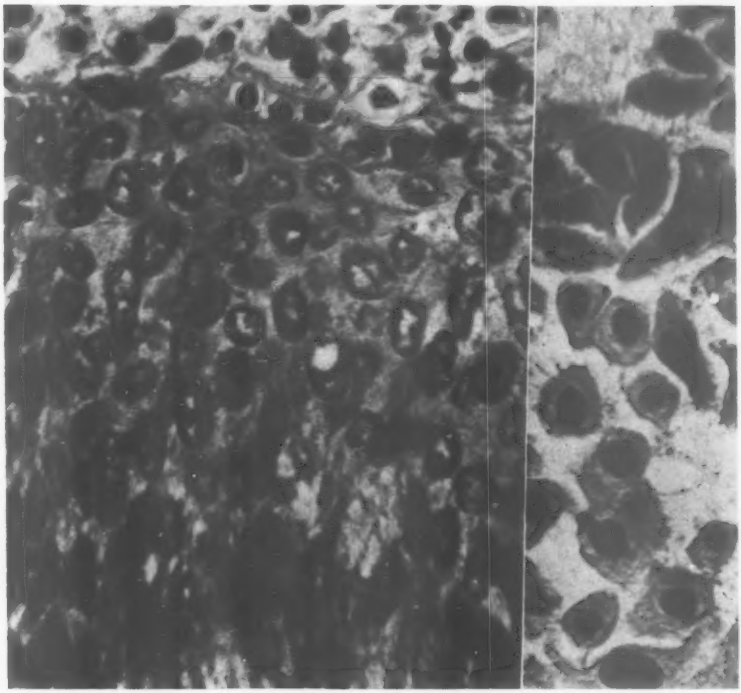


FIG. 2 demonstrates the orange-black cells of parakeratosis. It shows the "peeling off" of these cells of parakeratosis into the lumen of a gland as seen at the top of the photograph. It should be added that parakeratotic cells can also originate from areas of leukoplakia and are then associated with totally keratinized cells. This is an unusual pattern.

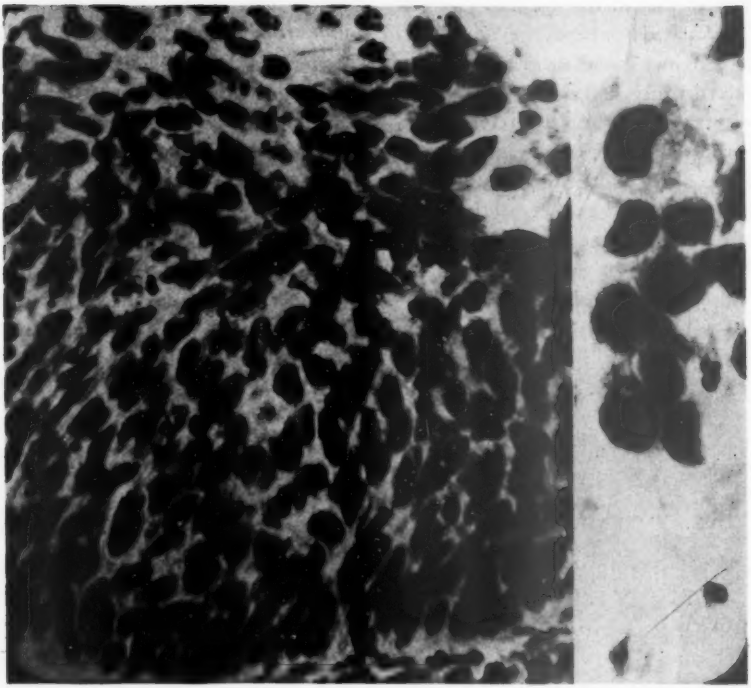
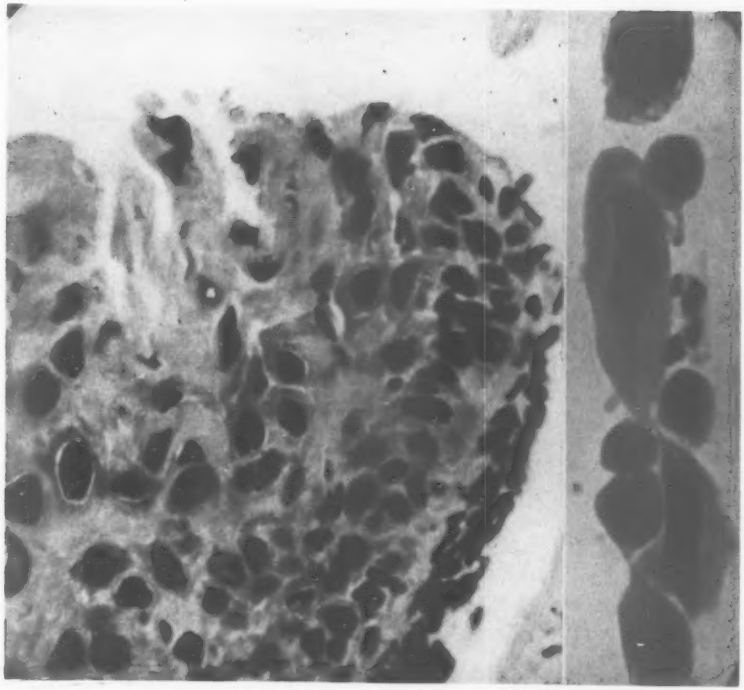


FIG. 3 is an example of a cluster of positive cells which can be exfoliated from either a carcinoma *in situ* or an invasive squamous cell carcinoma. In this cytological pattern type there is marked variation in size and shape of the malignant cells and their nuclei. The four-point cervical biopsy showed only a chronic cervicitis. Because this biopsy did not explain the exfoliated cells, a cold-knife conization was performed. Up in the endocervical canal, there was a rather extensive carcinoma *in situ*. Because of the large number of positive cells, one could expect the lesion to be extensive. The tissue photomicrograph shows one of the cervical glands replaced by this carcinoma *in situ*. The surface tumor cells show the same variation in size and shape as seen on the smear.

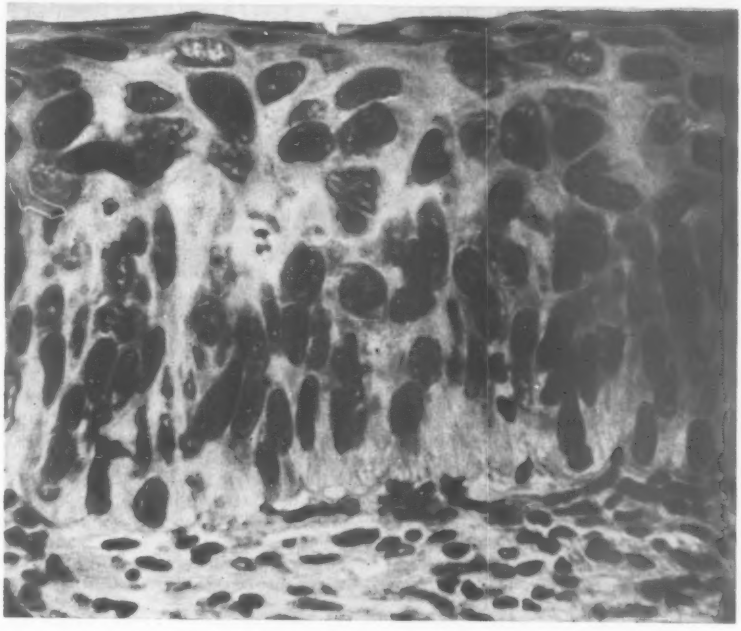
FIG. 4a is an unusual type of exfoliated cells presenting an entirely different appearance. These abnormal keratinizing cells somewhat resemble Papanicolaou's dyskaryosis group in that most of the cells have an abundant amount of cytoplasm, but differ in that the nuclei are extremely hyperchromatic. The tissue correlates well with the exfoliated cells, *i.e.*, malignant nuclei with abundant keratinizing cytoplasm. For those of you who would not interpret this lesion as malignant, the adjacent field has been photographed. This, also, is an unusual cell pattern.

FIG. 4b shows a difference in the two fields which may be merely one of degeneration of the malignant epithelium in the first field.

4a



4b



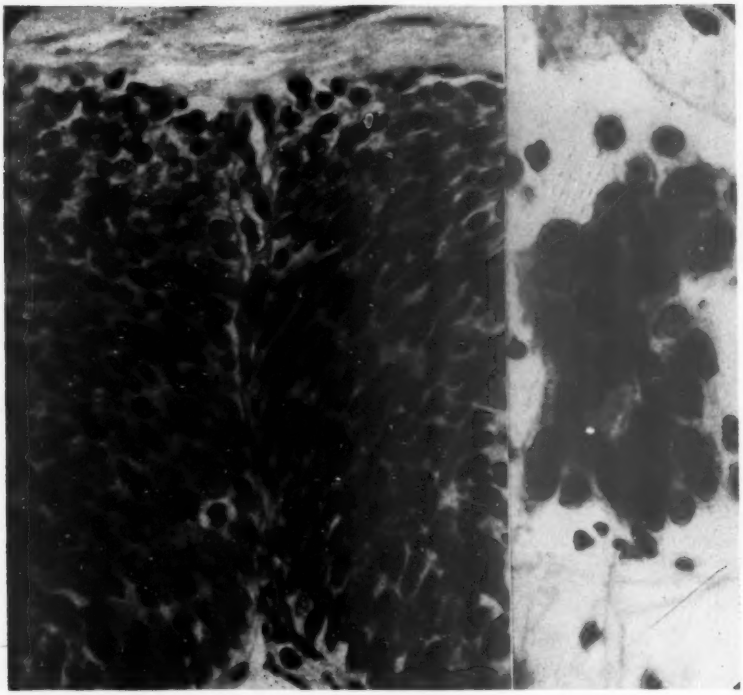


FIG. 5 demonstrates the small cell type of carcinoma *in situ*, also an unusual type. The positive exfoliated cells have two main distinguishing features; they are much smaller than any of the other cells demonstrated, though still hyperchromatic, and they occur almost exclusively in tight clusters so as to preclude good photographic reproduction. Tight grouping is an unusual feature of a smear from a carcinoma *in situ*. The tissue shows the same pattern.

Discussion

Clarice do Amaral Ferreira, Rio de Janeiro, Brazil: My experience with carcinoma *in situ* of the cervix is quite small. We have studied with care 60 cases of carcinoma *in situ* from the Institute of Gynecology, up to December, 1958, with the comparative results of cytology, colposcopy and histology. Of these 60 cases, only 36 were definitely carcinoma *in situ*, with semi-serial sections of the entire cervix; the diagnosis of the other 24 were based only on individual cervical biopsies.

I think it is still difficult to make a cytological diagnosis of carcinoma *in situ* from special cytological features. We can only suggest that it could be an "*in situ*" lesion.

Considering the opinions and experience of the main authors, I believe that possibly all of them are correct, each considering the problem from his own point of view. However, I feel that my opinion is closer to that of von Haam, who states: "We have found it (carcinoma *in situ*) easier to recognize in the smear than invasive carcinoma in which the cervical surface is often necrotic and does not contain many preserved cancer cells." Many times I have expressed this opinion in speaking about the diagnosis of carcinoma *in situ* and microcarcinoma.

I believe that it is difficult to recognize any typical cytologic picture of carcinoma *in situ*, as it is only a transition to the invasive form. Therefore, in such cases we find malignant cells, most often the differentiated squamous type, basal cells, very rarely the undifferentiated type, as Ruth Graham states: "usually in a clean smear, with preserved malignant, dyskaryotic or benign cells." There is also the presence of a certain number of dyskaryotic cells together with malignant cells, which we rarely find in cases of invasive carcinoma.

TABLE 1

Cell groups in smears	Carcinoma *in situ*	Invasive carcinoma	Early invasive carcinoma
1	2	8	0
1,2	6	15	0
1,2,3	10	5	4
2,3	7	1	2
2,3,4	53	4	2
1,2,3,4	3	14	7
Totals	81	47	15

Of the 36 definite cases of carcinoma *in situ*, 28 were grouped in Class IV (Papanicolaou) and six in Class III. The two false negative cases: one was associated with Trichomonas and one was a carcinoma *in situ* involving a gland, which was found only by histology after hysterectomy for myoma.

Olle Kjellgren, Gothenburg, Sweden: I agree with Ruth Graham that the typical cytological picture in carcinoma *in situ* is that of increased differentiation in contrast to the picture of invasive carcinoma, the predominating cell being the "third type malignant." We find the fiber cell to be very rare in carcinoma *in situ*, thus disagreeing with de Brux. Besides the "third type malignant" cells, there are nearly always present dyskaryotic cells from the parabasal and superficial layers.

Jule Kovacic and Srecko Rainer, Ljubljana, Yugoslavia: We have examined smears of 81 cases of carcinoma *in situ*. The atypical cells have been classified into four groups:

1. Atypical undifferentiated cells, which cannot be ascribed to any of the layers of the squamous epithelium.
2. Atypical cells that, owing to their morphological character, resemble those of the deep layer of the squamous epithelium.
3. Atypical cells that morphologically resemble the cells of the intermediate layer of the squamous epithelium.
4. Cornified atypical cells with a clearly distinguishable nucleo-cytoplasmic dissociation.

Among our cases of carcinoma *in situ*, we have been able to trace the cells of the first group alone only twice. In a smaller number they could have been observed in another 19 cases, but simultaneously with the cells from Groups 2, 3 and 4. In 53 cases there have occurred only cells of Groups 2, 3 and 4, those of Group 1 have hardly been found. Altogether the cells from Group 4 have been observed in 56 cases.

With the intention of ascertaining a possible difference, we have examined 47 smears of invasive carcinoma. Group 1 cells have been found in 42 cases, of which 23 contained only cells of Group 1 or of Group 1 and 2. We agree with Ruth Graham that smears of carcinoma *in situ* are mostly formed of differentiated cells. In all of our cases of invasive carcinoma, except in five, we have found undifferentiated cells but in the cases of carcinoma *in situ* we have, on the contrary, been able to find them in only 21 cases.

Checking the smears of 15 cases of early invasive carcinoma, we have had the impression that in their structure, these very much resemble those usually found in carcinoma *in situ*.

The results of our analysis are shown in Table 1.

According to our results we are obliged to think of the cytological indicator of an advancing malignant process as being more or less expressed dedifferentiation in cellular elements of orthotopic tissue. The cells of Group 2, 3 and 4 are decreasing in number as long as there is no gap that can be compared to "hiatus leukaemicus"; a phenomenon known in leukemias which indicates a malignant process.

The smears of carcinoma *in situ* combined simultaneously with an inflammation represent a difficulty in grouping. Such smears can simulate the smears of an advanced carcinoma. On the other hand, an advanced carcinoma with the histological pattern "carcinoma planocellulare cornescens" can to some degree resemble the smear of carcinoma *in situ*.

František Luksch, Prague, Czechoslovakia: On the basis of our experience, and in agreement with Ruth Graham, we are of the opinion that in smears taken from carcinoma *in situ* the number of differentiated cell-types predominates. However, we do not believe that this fact can be deducted from only one single property of the lesion, namely invasiveness. As is well known, especially to the histologist, a clear-cut distinction between carcinoma *in situ* and infiltrative carcinoma is often an unsolvable problem, and for that reason it would be inappropriate to draw a sharp cytological borderline between the carcinoma *in situ* and invasive carcinoma. For the cytological smear pattern is much less decisive in determining beginning infiltration than is the histological differentiation, the thickness of the necrotic layer, the character of the marginal covering and, last but not least, the size of the carcinomatous focus. In cases of large, necrotic and therefore poorly exfoliating, histologically immature cervical carcinomas, one will consequently expect less numerous and more

immature cells than in cases of differentiated carcinomas or carcinomas *in situ* without necrosis.

Therefore, we would be more interested in the frequency distribution curve by Ruth Graham (of undifferentiated cell types) (Fig. 3) looking like the abscissa carrying the groups "carcinoma *in situ*," "initial carcinoma" (approximately synonymous with Mestwerdt's "microcarcinoma") and "macrocarcinoma." We believe that a table supplemented in such a way could satisfactorily explain the apparent contradictions among the authors. One may predict, in this case, that the result should lie around a straight line which connects a minimum in the carcinoma *in situ* group with a maximum in the macrocarcinoma group. Thus the cytological borderline (50 per cent) may be better drawn — somewhat shifted to the right — in the middle of the second group (microcarcinomas) and not between carcinoma *in situ* and invasive carcinoma.

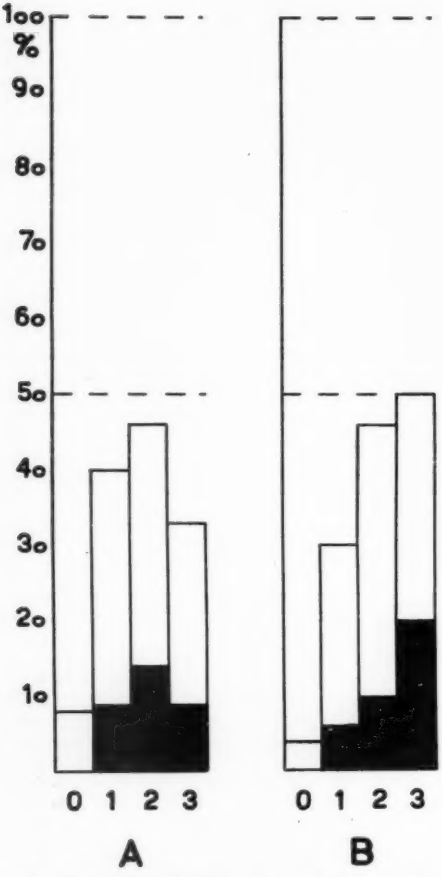


FIG. 1. Dyskaryotic cells. (0 = basal, 1 = parabasal, 2 = intermediate, 3 = superficial type.) Percentage of smears in A = 99 cases of carcinoma *in situ* and B = 50 cases of invasive carcinoma. Total height of columns = percentage of smears where the special type of cells was found. Black areas = percentage of smears where these cells were predominant.

Luis Montalvo Ruiz, Madrid, Spain: We agree with the criterion given by von Haam at the beginning of his paper. There are also basal and parabasal dyskaryoses in carcinoma *in situ* smears because of inflammatory reactions which very often accompany this type of carcinoma. The vaginal flora is not typical since we have seen healthy flora with abundant growth of Döderlein bacilli in carcinoma *in situ*, the same as in invasive carcinoma.

We agree with Ruth Graham: most of the carcinoma *in situ* cells belong to the third type differentiated cells, but this does not always happen, although we have seen undifferentiated cells, fiber cells and tadpole cells in many carcinomas *in situ*. For this reason we cannot accept a specific cytologic pattern in carcinoma *in situ*.

We also believe that, at times, the cytologic differentiation between a reversible dysplasia and carcinoma *in situ* is extremely difficult and a careful examination of the chromatin pattern under high power is necessary, such as is advised by de Brux.

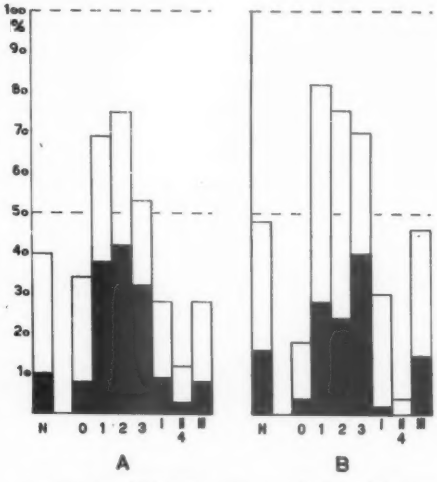


FIG. 2. Atypical cells. (N = naked nuclei, 0 = basal, 1 = parabasal, 2 = intermediate, 3 = superficial type cells. 4 I = fiber cells, 4 II = tadpole cells, 4 III = third type differentiated cells.) Percentage of smears in A = 99 cases of carcinoma *in situ* and B = 50 cases of invasive carcinoma. Total height of columns = percentage of smears where the special type of cells was found. Black areas = percentage of smears where these cells were predominant.

Jacqueline Mouriquand and Marcel Dargent, Lyon, Rhône, France: Our experience with the cytological features of carcinoma *in situ* (Queyrat's erythroplasia and Bowen's disease) agrees with that of von Haam: on the basis of cytology alone one cannot differentiate between carcinoma *in situ* and invasive carcinoma.

Like Ruth Graham, we find the majority of malignant cells to be of the third type. Undifferentiated malignant cells are not seen. Dyskeratotic cells with a more or less large orange-colored cytoplasm are very frequent.

The cytologic characteristics of invasive skin carcinoma are not quite the same as those of cervical carcinoma. Undifferentiated malignant cells without a clear-cut cytoplasmic border, such as described by Ruth Graham, are very uncommon. In basal cell carcinoma, third type malignant cells with cyanophilic cytoplasm, grouped in dense clusters, are to be seen. This type, in our experience, is found in Bowen's disease. In squamous cell carcinoma, isolated malignant cells with orange, dyskeratotic cytoplasm are seen. Such is the case in Queyrat's disease. True fiber cells are seldom seen.

Violette M. Nuovo, Paris, France: Like de Brux, I believe that the possibility of recognizing a carcinoma *in situ* is based on the presence of a great number of immature malignant basal cells in the smear.

I was very much impressed by Ruth Graham's work. Although we have never calculated the percentages of the different types of malignant cells encountered, we have always based the diagnosis of carcinoma *in situ* on the presence of cells of the second type which she describes (she states that she found more than 50 per cent of this cell type in 69 per cent of the carcinoma *in situ* cases, we have roughly the same results since we were able to diagnose roughly 70 per cent of the cases of carcinoma *in situ* according to the same criteria).

The only difference is the terminology: in my laboratory the cells described by Ruth Graham as "differentiated squamous cancer cells" are called "poorly differentiated or immature cancer cells."

Alfred Wacek and Jacques Jenny, Zürich, Switzerland: The smears of 99 cases of carcinoma *in situ* have recently been reviewed and compared to the smears of 50 unselected cases of invasive carcinoma. The cells were classified according to their differentiation. N means naked nuclei, O = basal cells, 1 = parabasal cells, 2 = intermediate cells, 3 = deep superficial cells, 4 = superficial cells. In the atypical cells, the last group was subdivided into I = fiber cells, II = tadpole cells and III = third type of differentiated cells. The absolute and relative amount of each type of cell, *i.e.*, normal, dyskaryotic and atypical cells, was roughly estimated. The nonspecific changes such as hemorrhage, leukocytes, histiocytes, etc., were also noted.

According to the grading of Papanicolaou, the cervical smears of the 99 cases were grouped as follows: 2, Class II; 16, Class III; 56, Class IV and 21, Class V. One smear was technically unsatisfactory and therefore could not be classified.

The distribution of the dyskaryotic cells according to the classification given above is shown in Figure 1. The total height of the columns gives the percentage of smears where the special type of cells was found. The black areas indicate the

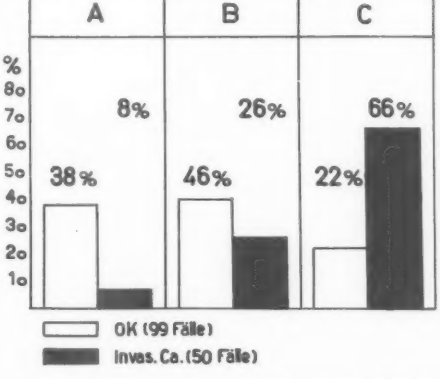


FIG. 3. Relationship of normal to pathological cells in 99 cases of carcinoma *in situ* (OK) and 50 cases of invasive carcinoma. A: Percentage of cases with mostly normal cells. B: Percentage of cases with nearly equal amounts of normal and pathological cells. C: Percentage of cases with mostly pathological cells.

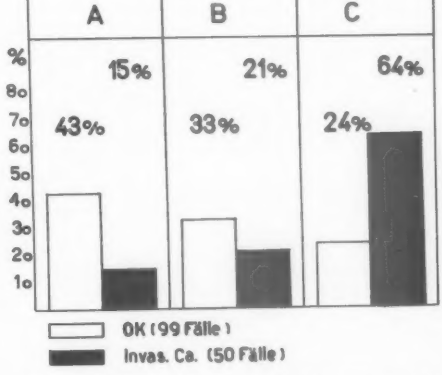


FIG. 4. Amount of leukocytes, histiocytes and red blood cells in 99 cases of carcinoma *in situ* (OK) and 50 cases of invasive carcinoma. A: Few such elements, so-called "clean" smears. B: Moderate amount of such elements. C: Large amount of such elements, so-called "dirty" smears.

percentage of smears where these cells were predominant. It can be seen that 40 per cent of the smears of carcinoma *in situ* (A) showed dyskaryotic basal cells (1), whereas in invasive carcinoma, these cells were found in only 30 per cent of the cases. Dyskaryotic superficial cells (3) were found in 32 per cent of the cases of carcinoma *in situ* and in 50 per cent of the cases of invasive carcinoma. Therefore, there seems to be a slight shift towards higher differentiation in invasive carcinoma, which would be in opposition to the opinion of Ruth Graham. However, the difference might be statistically insignificant, since the method is based on estimation.

The atypical cells, when classified in the same manner, show the distribution noted in Figure 2. There is no gross difference in cellular differentiation between carcinoma *in situ* and invasive carcinoma. Most of the cells originate in the parabasal and intermediate layers. Slight variations are again within the limits of the method.

We conclude from this, in agreement with von Haam, that it is hardly possible to differentiate between carcinoma *in situ* and invasive carcinoma from the pathological cells alone.

However, there is a difference in the amount of pathological cells in relation to normal cells, as shown in Figure 3. In most of the cases of carcinoma *in situ* the pathological cells are outnumbered or equalled by the normal ones, whereas in most cases of invasive carcinoma the pathological cells are dominant. A similar relationship is found in Figure 4 where the amount of leukocytes, histiocytes and red blood cells is noted. Here again, there is a marked difference between carcinoma *in situ* and invasive carcinoma. We agree with von Haam, that by these criteria the experienced cytologist might diagnose the underlying pathological lesion.

Closing Remarks

Ruth M. Graham: There is surprising agreement among the discussants that the exfoliated cells from carcinoma *in situ* are more differentiated than those from invasive cancer.

Luksch asks for a distribution curve for which I am grateful. The Table below is probably a better way of presenting my data than the chart in the main paper of this section.

This Table indicates that 55 of the 105 cases of carcinoma *in situ* could have been diagnosed accurately on the basis of over 80 per cent of the malignant-cell population being classified as the third-type differentiated cell. Only three cases of the 55 cases would have been in error (5%).

However, the tabulation also indicates that there is a large group of cases where it would not be possible with any accuracy to distinguish carcinoma *in situ* from invasive carcinoma.

Edward E. Siegler: Those who write, "On the basis of cytology alone one cannot differentiate between carcinoma *in situ* and invasive carcinoma," should re-investigate their criteria, since some of us have not found this true in our own laboratories.

It has been our experience that in cervical carcinomas we can predict in about 80 per cent of the cases whether the tissue will be invasive or not. In a positive smear of a patient in late pregnancy, for example, this can be important.

With but a few relatively minor changes we have for about 10 years used the pure Ruth Graham third-type cell pattern as indicative of carcinoma *in situ* and have been very happy with the results. A Papanicolaou Class V pattern with a very clean background is more of a problem because occasionally the tumor is only *in situ*. One other helpful point is the age of the patient: the younger the patient, the more likely is the diagnosis to be carcinoma *in situ*.

TABLE 1

Percentage of third-type differentiated cells	Number of cases	Carcinoma *in situ* and percentage	Invasive carcinoma and percentage
100%	30	30 = 100%	—
80	25	22 = 88	3 = 12%
60	26	15 = 58	11 = 42
40	55	16 = 29	39 = 71
20	68	12 = 18	56 = 82
1	84	9 = 13	75 = 87
0	4	1	3

Fluorescence Microscopy on Exfoliated Cells of Carcinoma *In Situ*

B. Cornelis Hopman*

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A larger amount of DNA as well as RNA is characteristic of cancer cells. Although in normal cells the amount of diploid nuclei within a species is constant, in cancer, increase of chromatin is found due to the polypoid characteristics of the nucleus. The methyl green pyronin[1] as well as the Feulgen staining may demonstrate such increased DNA content.

The amount of RNA in growing embryonic and malignant tissue is likewise increased. The methyl green pyronin staining is often able to show this very effectively.[1] But also photometry after hematoxylin and eosin[2] and spectrophotometry[3] can show these effects.

Fluorescence microscopy has been used in increasing frequency in recent years. It can be used for living as well as for fixed tissues and the method may give polychrome pictures of great brillancy. Mellors and Silver used it for fluorometric scanning with an automatic registration of the increased chromatin content of the nuclei.[4] Bertalanffy[5] uses acridine orange (AO) as staining material. The method differentiates two nucleic acids. RNA in the cytoplasm and nucleolus gives red fluorescence. DNA of the nucleus gives green fluorescence. Superficial cells of the cervical smear show a green cytoplasm with a greenish-white nucleus. Intermediate cells display a larger nucleus and reddish-brown cytoplasm. Basal cells have a brick-red cytoplasm with a greenish-white nucleus. Red blood cells are not stained, an advantage of the method. Leukocytes show a whitish-green fluorescence of the nucleus. Mucus stains whitish-green. Trichomonas vaginalis has a red-orange cytoplasm and yellow nucleus. Bacteria give an orange-red background to the slide picture.

Cancer cells stand out in brilliant colors with the cytoplasm in flaming red-orange. The nuclei are yellow-green with yellow hues and are enlarged. The nucleoli are orange-red as is the cytoplasm. The nuclear membrane is thickened and shows an outstanding white-yellow color. It often has an irregular outline. Bertalanffy, who introduced the method for cytology, got the same good diagnostic results with this staining technic as with the Papanicolaou staining method. The average screening time is only three minutes and the method is useful for mass screening.

The author examined the method in 20 cases of cervical cancer of different stages and 100 normal cases. The method has important advantages but also certain disadvantages. The advantages are:

1. A simple inexpensive apparatus. We used a fluorescence lamp from the American Optical Co. with bulb and transformer, filter holder, a Corning blue filter 3 x 3 x ½" #5113 and a Kodak Wratten G. filter cut to desired size in the eye pieces of the microscope.
2. The staining procedure is simple and takes only six minutes. The method is described in *Cancer*.[5]
3. Red blood cells, an important obstacle

* This work was supported by a research grant from the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

in reading Papanicolaou preparations, are not stained.

4. The method is of high scientific order. It is a cytochemical method based on staining of different nucleic acids.
5. The average screening time is only three minutes. The demands on the skill of technicians can be reduced because the colors of malignant cells stand out so distinctly against normal cells that they are immediately visible.
6. It can be used for mass screening.

There are certain disadvantages:

1. Cancer diagnosis, as generally applied by the Papanicolaou staining technic, uses the nucleus rather than the cytoplasm as diagnostic criterium. This technic gives a clear distinct picture of the chromatin of the cell with all its delicate qualities as: quantity of chromatin, fine or coarse clumping, division of chromatin, color intensities of chromatin, etc. In my opinion these qualities are of essential significance in the cytologic cancer diagnosis for differentiation not only of benign and malignant cells, but also for the different stages of malignancy. I do not find these same qualities present in malignant cells stained with acridine orange for fluorescence microscopy: The nucleus is yellowish-green, sometimes with yellow streaks. Of considerable help in cancer cells is the nuclear membrane, standing out as a white-yellow wall surrounding the nucleus and showing its increase in size and irregular outline. Still these characteristics do not compensate entirely for the loss of the delicately outlined chromatin structure as seen in Papanicolaou slides.
2. Basal cells, Trichomonas vaginalis and endocervical cells are also red or orange-red. These structures have to be differentiated from malignant cells. This can be done by the nuclear size and structure, but here again the delicate characteristics of the chromatin picture is of essential importance.
3. When cells degenerate, the cytoplasm often degenerates more intensely than the nucleus. In cytolysis we often find degenerated cytoplasmic fragments but still intact naked nuclei. Degenerated cancer cells often display a chromatin picture distinct enough to recognize them as malignant cells. When these cells are stained with acridine orange one misses their most important staining structure: the cytoplasm.
4. Cancer cells, in a very early stage of cancer, show most of their characteristics in the nucleus, not in the cytoplasm.
5. Cancer cells, in a very advanced stage of cancer, often show only a slight rim of cytoplasm or no cytoplasm at all. Here the diagnosis with acridine orange is again more difficult than with the Papanicolaou staining method.

Summarizing, I believe in the importance of the fluorescence microscopy as a scientific cytochemical method as described by Bertalanffy. I hope that it also can be used as a screening method. My experience up to now, however, has shown certain disadvantages of the method in degenerated cancer cells, and in very early and very advanced cancers.

Bibliography

1. Hopman, B. C.: Mikroskopie. **10**: 251, 1955.
2. Foraker, A. G.: A.M.A. Arch. Path. **53**: 250, 1952.
3. Moberger, G.: Acta radiol. suppl. **112**: 1, 1954.
4. Mellors, R. C. and R. Silver: Science. **114**: 356, 1951.
5. Bertalanffy, L. V., M. Masin and F. Masin: Cancer. **2**: 873, 1958.

Can Carcinoma *In Situ* Be Differentiated from Invasive Carcinoma by Means of Exfoliative Cytology?

JEAN A. DE BRUX, JACQUELINE DUPRÉ-FROMENT
Paris, France

As we have seen, it is difficult to differentiate carcinoma *in situ* from the active undifferentiated metaplasia (hyperplasia of the reserve cells), and relatively difficult to differentiate it from the irregular dysplasias.

But in the case of the differentiation between carcinoma *in situ* and the invasive carcinoma, the situation is somewhat different, for, whereas the immature invasive carcinoma is easily distinguished, it is rather difficult, on the contrary, to differentiate the mature epidermoid carcinoma.

A. The immature invasive carcinoma presents only immature elements, most often identical, grouped in patches or strips of contiguous cells. These cells have a vaguely parabasal aspect, with exclusively cyanophilic cytoplasm. The nuclei are more polymorphous, with irregular or notched contours. The aspect of the chromatin varies from one cell to another; it may be either:

1. in dense, irregular clumps, with distribution similar to that in carcinoma *in situ;* or
2. sparsely scattered, reduced to a few bulky masses; or
3. of homogeneous aspect, but as if erased or wiped out.

Anomalies are but rarely noted, but inflammation and necrosis are frequent.

These facts suffice to easily differentiate carcinoma *in situ* from the immature invasive carcinoma.

B. The mature or moderately mature epidermoid carcinoma presents greater difficulties than the form just described (less perplexing, however, than with certain irregular dysplasias).

The elements found are rather diversified, in form and size, as well as in the nuclear and cytoplasmic characteristics.

The mature abnormal elements occur with much greater frequency than in carcinoma *in situ:* cornified cells of superficial and intermediate type, fiber cells, and cornified basal cells having, however, a very large nucleus of dysplastic rather than carcinomatous type.

The immature elements are of parabasal type, with festooned nuclear contours, and chromatin either in dense clumps, irregularly distributed, or in large masses with interstitial vacuoles.

Although in rare cases some errors have been made, in general the diagnosis of carcinoma *in situ* is relatively easy. But is the carcinoma *in situ* really an entity?

JORGE CAMPOS R. DE C.
Lima, Peru

THERE are no cytologically characteristic features which would allow the diagnosis of carcinoma *in situ* with certainty by purely cytological means. However, in most cases the cervical cytology is highly suggestive and allows the cytologist to indi-

cate the possible presence of carcinoma *in situ*. The final diagnosis should always be made by histology.

The cells exfoliated from a carcinoma *in situ* are commonly found isolated; they have a medium size with an average diameter of 20 to 25 μ with round or oval form; the edges are neat, cytoplasm is scarce and cyanophilic, without vacuoles or leukocytic infiltration. The nuclear-cytoplasmic ratio is disturbed. The nuclei are oval or round, vesicular with an average diameter of 12 to 15 μ; the nuclear chromatin is finely granular, deeply stained. Nucleoli are rarely seen.

In addition to the above characteristics, the smear of carcinoma *in situ* shows cellular isomorphism, absence of bizarre or "tadpole" cells and absence of cornified material or cellular detritus.

B. Cornelis Hopman*

Miami, Florida, U.S.A.

In situ carcinoma and invasive carcinoma are pathologic findings and conceptions. *In situ* carcinoma is a deviation where the cancerous process is limited to the squamous cell layers without infiltration into the sub-epithelial tissue. It represents the initial stage of squamous cell carcinoma. Later invasion may take place with perforation through the basal membrane, infiltration of the subepithelial tissue and penetration into blood and lymph vessels, causing metastases.

In histology we consider loss of polarity when the cells show an irregular arrangement with their long axis not perpendicular to the surface of the epithelium. Grading of tumors in pathology to define the degree of malignancy is made by examination of the degree of anaplasia, loss of differentiation of the cells, the degree of hyperchromatism and the number of mitotic figures.

In cytology we consider the cells, their degree of differentiation, the relation between nucleus and cytoplasm, chromatin clumping and content, the characteristics of the nucleolus, variations in form and size of the nucleus, eosinophilia and cyanophilia. The secretions, red and white blood cells, mucus and bacterial content influence our diagnosis. The number of cells, especially the number of squamous cells compared to the number of white blood cells, gives important data in respect to prognosis of radiated cervical cancer. The number of tumor cells compared to the number of red and white blood cells gives data in respect to the grading of the cancerous process in consideration.

The cytologist does not find cell layers and cell membranes, blood or lymph vessels, but an irregular, disorderly mass of cells of all layers, partly over-lapping and mixed with all sorts of secretions. He tries to localize and recognize the cells and reconstruct the original histologic structure as a watchmaker reconstructs a watch from all sorts of little gadgets.

From the foregoing it is clear that it is impossible to differentiate carcinoma *in situ* from invasive carcinoma by means of exfoliative cytology. However, though cytology is unable to draw the exact line where carcinoma *in situ* ends and invasive carcinoma begins, there are important characteristics in the slides showing the

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degree of the cancerous process. The cytologist seldom sees mitotic figures, but the differentiation of the cell, the relation of the size of nucleus and cytoplasm, the amount and division of the chromatin in the nucleus, the size and character of nucleoli, the number of cancer cells present, their arrangement on the slides, the presence and relative amount of secretions give the cytologist at least an approximate idea of the degree of malignancy. The degree of increasing malignancy as seen in histologic slides is approximately as follows:

1. normal cervix, cervicitis;
2. cervicitis, atypia of cells, some loss of polarity;
3. basal cell hyperplasia;
4. carcinoma *in situ;*
5. invasive carcinoma with its four different gradations.

In cytology this is more or less in accord with the stages of Papanicolaou I, II, III, IV and V.

One hundred cases were examined cytologically and histologically, approximately 20 cases in each stage.

Stage I of Papanicolaou shows normal cells. There may be some infection visible from the number of leukocytes. It is important to ascertain that a greater or lesser degree of infection, especially when combined with Trichomonas vaginalis, is accompanied by slight deviations of the nuclei of the squamous cells. We see a small halo around the nucleus and a slight irregularity of the border of the nuclear membrane, but these deviations are minimal and are regarded as within normal limits. This is Stage I where the cytologist reports negative for cancer. He may add that infection is present. The pathologist gives a negative report, maybe cervicitis.

Stage II shows deviations of slightly larger degree. There is some hyperchromatism, there may be a slight irregular division of chromatin in the nucleus, the halo is more pronounced, the nuclear membrane may show slight indentations. However, the nucleus is still within normal limits of size and form and there is no deviation of the normal nuclear-cytoplasmic ratio. This is Stage II where the cytologist reports negative for cancer, advises repeat smear in three months. The pathologist makes the diagnosis of negative for cancer, or cervicitis with slight atypism of cells.

Stage III shows definite deviations. Here the normal nuclear-cytoplasmic relations are interrupted. The nucleus is too large for the size of the cell, shows distinct hyperchromatism with irregular chromatin division and coarse clumping, irregular nuclear border with indentations, halo formation, enlarged nucleolus. We consider coarse clumping of chromatin, irregular division of chromatin and enlarged nucleolus as the most important stigmata of beginning cancer. What these deviations still distinguish from real cancer cells is a high

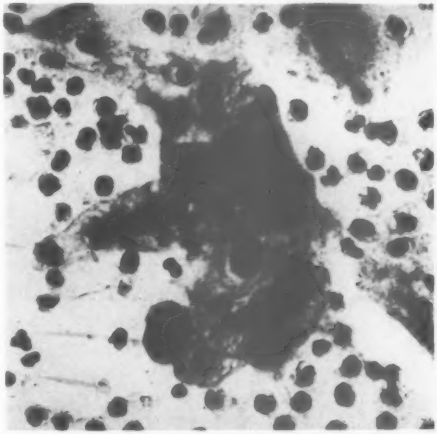

FIG. 1a. Cytology slide: Stage III, dykaryotic cells. Nuclei of the cells display malignant features such as enlargement, hyperchromatism, coarse clumping of chromatin, some prominence of the nucleolus. There is disruption of the normal nuclear cytoplasmic ratio. However, there is still abundant cytoplasm around the nucleus. Compared with the normal cells to the left the deviations are moderate (x581).

FIG. 1b. Histologic slide of the same patient: basal cell hyperplasia. There is loss of polarity of the basal cells which appear to be becoming restless. They are not confined to the basal cell membrane but rise up to more superficial areas without reaching the epithelial surface. There are distinct nuclear changes of the cells (x115).

degree of differentiation of the abnormal cells. Although the nucleus may show definite signs of beginning cancer, there is still so much cytoplasm around the nucleus that no definite cancer diagnosis can be made. The number of abnormal cells is relatively low. This is Stage III where the cytologist makes the diagnosis of dyskaryotic cells[1-5] (Fig. 1a) and leaves to the gynecologist the decision to make a biopsy or to perform follow-up cytologic examinations in expectation of the course of events. The clinician will judge the patient according to aspect of the cervix, age, number of children, etc. In younger childless patients he will be more conservative, in older patients who have already borne their children he will be more aggressive. The Stage III is the borderline stage. The pathologist often makes the diagnosis of basal cell hyperplasia or cell atypism (Fig. 1b). In the statistics of the P.C.R. (Positive Cytology Registry of the Department of Obstetrics and Gynecology)[6] 14 per cent of Stage III patients proved to have cervical cancer.

Stage IV shows definite cancer cells. The nuclear-cytoplasmic ratio is distinctly changed in the direction of the nucleus. Besides the large size of the nucleus there is pronounced hyperchromatism, prominent nucleolus, variation in size and form of the nucleus, coarse clumping of chromatin. The number of malignant cells also is larger than in Stage III. The cytologist makes the diagnosis of positive for cancer cells with advice to make a biopsy (Fig. 2a). The pathologic diagnosis in these cases is often carcinoma *in situ* (Fig. 2b), but this stage also contains some invasive carcinoma.

Stage V shows definite cancer cells, as Stage IV, but here the number of malignant cells is larger, the deviations are more

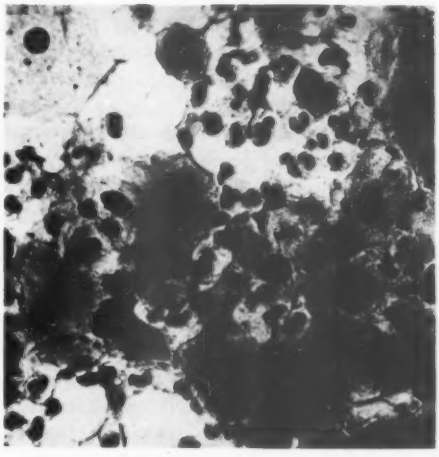

FIG. 2a. Cytologic slide: Stage IV, positive for cancer cells. Compared with Fig. 1a (same magnification) the changes are more marked. The nuclei are larger, the chromatin more clumped, the nucleolus more conspicuous, the cytoplasm smaller in amount. There is more difference in form and size and chromatin content of the nuclei. The cells at the bottom are extremely hyperchromatic and more anaplastic than the others. In comparison with the normal cell, upper left, the changes are distinctly more pronounced than in Fig. 1a (x581).

pronounced. Hyperchromatism, coarse clumping and prominent nucleoli are very striking. There is excessive variation in form and size of the nucleus. There are cells with multiple nuclei, multilobulation, gigantic forms of cells and such abnormal cytoplasmic formations as "tadpole cells," "fiber cells," "snake cells," etc. The cytologist makes the diagnosis of positive for cancer with advice to perform a biopsy (Fig. 3a). The pathologic diagnosis is often invasive carcinoma (Fig. 3b) but this stage also contains some cases of carcinoma *in situ*.

There is still a further development observed in cytology, the stage of the far advanced cervical cancers. Here a decrease of differentiation of the cell has taken place which is very characteristic. The clinical diagnosis is often cauliflower tumor, fungating cancer, crater form of cancer or far

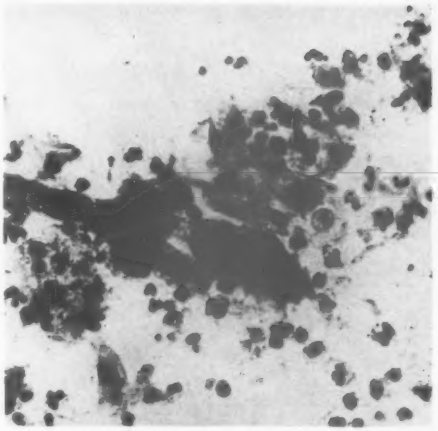

FIG. 3a. Cytologic slide: Stage V, positive for cancer cells. With the increasing grade of cancer the nuclei become larger and more hyperchromatic, the nucleoli more pronounced. Coarse clumping of chromatin is marked. The amount of cytoplasm is again smaller, though an occasional differentiated cell is encountered. Throughout the cancerous process the infection is extensive, cocci are visible with high magnification, Döderlein bacilli or other acid forming bacilli are not present. Normal cells often show eosinophilia and pyknosis (x581).

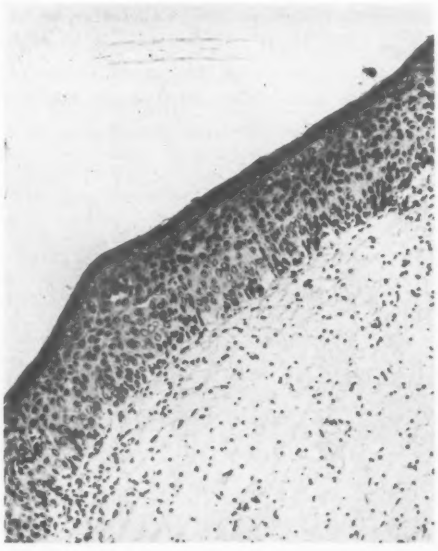

FIG. 2b. Histologic slide of the same patient: carcinoma *in situ*. There is loss of polarity, anaplasia, and there are nuclear changes in the cells. The abnormal cells have reached or nearly reached the surface, but there is no invasion of the subepithelial tissue (x115).

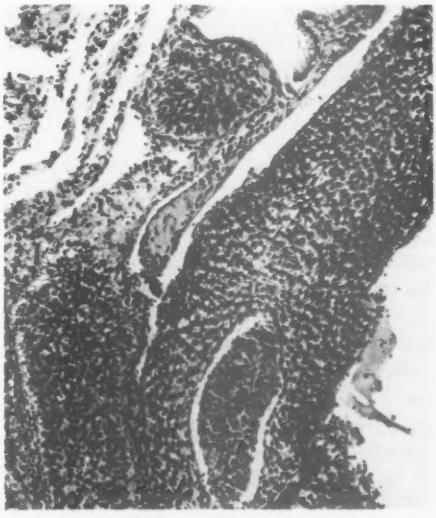

FIG. 3b. Histologic slide of the same patient: invasive cancer. Sheets of malignant cells invade into the subepithelial tissue and around the cervical glands. There is extensive round cell infiltration of the stroma. The cells are more anaplastic than in 2b (x115).

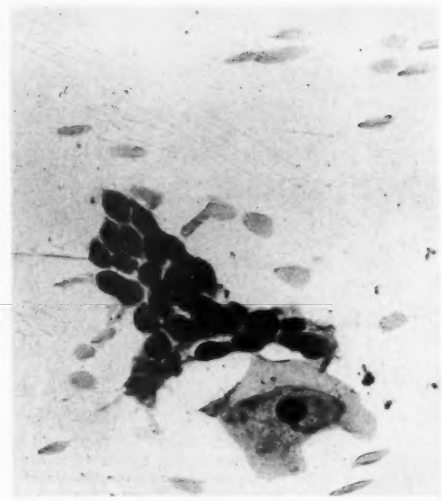

FIG. 4a. Cytologic slide: Stage V, positive for cancer cells. Far advanced cancer. The cells become extremely anaplastic. There is only a small rim of cytoplasm or no cytoplasm at all. The nucleus is shrunken, very hyperchromatic but often scarcely larger than the nucleus of the normal cell below. There is still variation in form and size, but due to the decrease in general size the differences are hardly visible. The malignant cells seldom appear in clumps, are often single and frequently covered with red and white blood cells. This advanced form of cervical cancer is often missed by the cytologist. Malignant cells often are not present, or the cytologist may take these small malignant cells for monocytes or endocervical cells (x581).

advanced cancer with bleeding. These cases can be easily missed by cytology because of the extensive infection and bleeding which often accompany these far advanced cases, cancer cells are rinsed away and present in only small numbers or covered with blood and leukocytes. Due to the high degree of malignancy the cells show only slight differentiation, are surrounded by a minimal rim of cytoplasm or no cytoplasm at all. They frequently appear singly or only in tiny clumps. They are often small and if not well observed may appear as monocytes and are diagnosed as such. The few malignant cells may be covered by a large amount of red or white blood cells making their recognition still more difficult. The cytologist often makes the diagnosis: no cancer cells seen but only a few cells on the slides. Advise repeat smears. The next smears frequently reveal the state of affairs (Fig. 4a). An increase of the grade of cancer is often accompanied by an increase of eosinophilia and pyknosis of the normal cells with an increase of leukocytes and rising *p*H. Cocci are frequent; Döderlein bacilli and other lactic acid forming bacilli are uncommon. The histologic slide shows a high degree of invasion, extensive hyperchromatism of cells, low grade of differentiation and a high degree of mitosis. The pathologist makes the diagnosis of invasive cancer, Grade III or IV (Fig. 4b).

Summary

The exact staging of cervical cancers by cytology is not feasible because the criteria which are the foundations of this staging are of pathologic nature. Cell layers, polarity of cells, basal cell membranes, connection of the malignant cells to the surrounding tissue and infiltration are not seen in

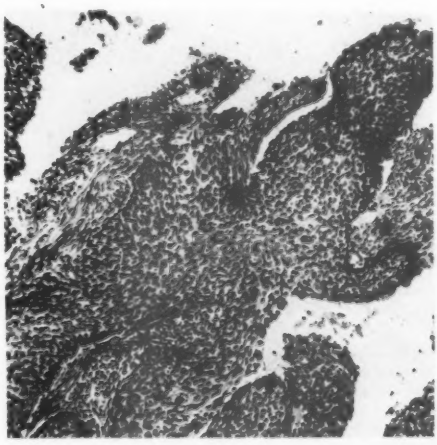

FIG. 4b. Histologic slide of the same patient: invasive carcinoma Grade III. Extreme invasion of malignant cell sheets, extensive hyperchromatism, anaplasia, many mitotic figures (x115).

cytology. However, in cytology it is possible, especially by examination of the nuclear-cytoplasmic relation, the nature of the malignant characteristics of the cells, the degree of differentiation and dedifferentiation in far advanced cancers, to have some definite knowledge of the degree of malignancy. The number of abnormal cells, their grouping, the cervical secretions and bacterial contents also play a role in this recognition.

Bibliography

1. Fox, Clifford H., D. E. Smith and W. N. Thornton: Transactions Inter-Society Cytology Council, Nov., 1957.
2. Koss, Leopold G. and G. R. Durfee: Transactions Inter-Society Cytology Council, Nov., 1957.
3. Papanicolaou, G. N.: Atlas of Exfoliative Cytology. Cambridge, 1954, Harvard University Press.
4. Papanicolaou, G. N.: Ann. Int. Med. **31**: 661, 1949.
5. Nieburgs, H. E.: Transactions Inter-Society Cytology Council, Nov., 1957.
6. Ferguson, J. H. and D. Cavanaugh: Am. J. Ob. & Gyn. **76**: 773, 1958.

Discussion

Anthony F. Anderson, Edinburgh, Scotland, U.K.: It seems to me, as always, that those who offer to distinguish the two are indulging in guesswork, and even if they are 90 per cent successful (accurate is too strong a word) it is entirely unnecessary, for a biopsy must be made. De Brux's final question left me breathless! Campos puts it more the way we see it, but it is still not necessary to suggest it, and it is because it is suggested weakly or strongly by so many that this question continues to fester. Hopman lends point to this belief where he states that some carcinomas *in situ* can be found following Stage V cytology reports.

Friedrich Bajardi, Graz, Austria: We agree with the reports of de Brux, Dupré-Froment and Campos. Exfoliative cytology permits certain conclusions as to the existence of both a carcinoma *in situ* and advanced invasive carcinoma of the uterine cervix. However, only histology can make the respective diagnoses with certainty. Some cases of very early invasion are not distinct from carcinoma *in situ* as far as the cytological picture is concerned. Diagnoses based solely on exfoliative cytology would thus lead to significant errors.

Hanns-Werner Boschann, Berlin, Germany: In daily cytologic practice the cytologist often feels himself tempted to move away from the restricting diagnosis "Class V, consistent with malignancy" and to give further details, *e.g.*, "apparently carcinoma *in situ*" or "consistent with invasive carcinoma." This differentiation is based on the general aspect of the smear (leukocytes, erythrocytes, cell debris, "clean" or "dirty" background), and on the occurrence of characteristic cell types (dyskaryotic and more or less differentiated cancer cells). The description of the criteria for the differentiation of pre-invasive from invasive carcinomas is more difficult than their application in practice and most cytologists agree about the diagnostic principles. In the great majority of cases the cytological differential diagnosis will agree with the result of the biopsy. Nevertheless, there are inevitable sources of error, *e.g.*, if the smear had been collected only from the noninvasive marginal area with an invasive carcinoma elsewhere. Moreover, carcinoma *in situ*, as well as invasive carcinoma, may offer different degrees of maturation, so that some invasive types may yield a picture of "*in situ*" while pre-invasive types may look like "invasive," especially if their surface has been injured or if almost all layers of a carcinoma *in situ* have been shed by mechanical or inflammatory influences.

For these reasons the cytologist should be reserved in his attitude towards the cytologic differentiation of invasion. He should register this subtle diagnosis for himself, for his own training, but should, in my opinion, confine his diagnosis for the clinician as "consistent with malignancy." A correct cytological differentiation would not relieve the clinician from the obligatory histological control, while a cytological error would diminish the cytologist's prestige, especially in the minds of clinicians who are not familiar with the basic principles of cytologic diagnosis.

For my own studies in differentiating carcinoma *in situ* from invasive carcinoma by means of cytochemistry, I would like to refer to Acta Cytologica, **1**: 30, 1957; **2**: 250, 1958; and Transactions of the Sixth Annual Meeting of the Inter-Society Cytology Council, p. 10, 1958.

Clarice do Amaral Ferreira, Rio de Janeiro, Brazil: We agree with Campos and Hopman (at least to the limits of our experience) that cytology can make a provisional diagnosis of carcinoma *in situ*. The definitive diagnosis is of course a histological one. There is still a lack of extensive cytological experience in these cases. Nevertheless, the cytologist has the possibility of suggesting the existence of a carcinoma *in situ*. We have done it several times and in many instances we were

correct. Our criteria is first of all a feeling that cannot be translated into words; and secondly, the presence of malignant cells, basal type, in a clean smear, together with many dyskaryotic cells. Sometimes the diagnosis of this condition is easier cytologically than that of advanced invasive carcinoma.

Ruth B. Graham, Buffalo, New York, U.S.A.: I would agree with de Brux that it is easier to distinguish an immature invasive carcinoma from a carcinoma *in situ* than it is to distinguish the mature invasive carcinoma. However, the overlap is considerable even with the immature invasive carcinoma. For this reason, I would agree with Campos that the diagnosis cannot be made with certainty cytologically. The diagnosis of carcinoma *in situ* is not a cytologic diagnosis. It is a histologic diagnosis.

Hopman's thesis that as the tumor becomes more advanced the type of malignant cell becomes more undifferentiated is an interesting one. While this may be true in a general way, there is such individual variation that to try and predict the clinical extent of the disease by the cytologic picture is not feasible in my opinion.

I would like to comment on another statement of Hopman's though it does not pertain to the subject under discussion. Authors fairly often state, as Hopman does here, that cancer cells are difficult to find in advanced cancer. I do not agree with this statement. In 24 consecutive cases of Stage IV carcinoma of the cervix, cancer cells were found in 22. In 15 of these cases malignant cells comprised more than 10 per cent of the epithelial cell population.

Jacques Jenny and Alfred Wacek, Zürich, Switzerland: The individual cells from a carcinoma *in situ* (Oberflachencarcinom) are as a rule better preserved, exhibit less autolytic changes and exfoliate more frequently as single cells than cells from an invasive carcinoma. Cells from the latter are often poorly preserved, exhibit a great degree of autolysis and lie in groups. The smear pattern from a carcinoma *in situ* displays a certain uniformity, whereas the picture of invasive carcinoma is more irregular and varied. The nucleoli are present in higher numbers and are also better developed than in carcinoma *in situ*. The chromatin network is generally coarser in invasive carcinoma. However, there does not seem to be a statistically significant difference between the two forms of carcinomas, either in regard to the nuclear size or in the nuclear structure. It is our belief that from the presence of more or less mature cells in the smear, differential diagnostic conclusions as to the distinction between carcinoma *in situ* and invasive carcinoma should not be drawn too liberally.

With invasive carcinoma we usually find quantitatively more pathological cells than with carcinoma *in situ*. This phenomenon may be explained by the fact that the lesions of invasive carcinoma have a greater surface area than carcinoma *in situ* and the number of exfoliated cells may be assumed to be proportional to the surface area involved and the desquamation tendency of invasive carcinoma is surely higher than with carcinoma *in situ*. Smears from carcinoma *in situ* are usually clean or only moderately "dirty" whereas in invasive carcinoma some of the smears contain enormous amounts of "concomitant" elements which often render the evaluation more difficult.

We do not believe that in the individual case the question of invasiveness can be answered from the cytological picture alone, even though the above criteria may give some hints to the experienced cytologist. From the practical standpoint this question is not even of great importance, since the definite diagnosis has to be made anyway by histological examination.

Maria Kawecka and Hanna Starkiewicz, Gliwice, Poland: The value of exfoliative cytology consists mainly in detecting cancer in women with erosion, or among apparently healthy ones.

Whenever an advanced invasive cancer is found, biopsy is performed before treatment which makes cytology unnecessary. However, inasmuch as early cervical carcinoma reveals certain cytologically characteristic features, we have compared results of the cytological examination of 165 cytologically diagnosed cases of carcinoma *in situ* with their histological evaluation. We found in this group of cases that the pathologist made the diagnosis of carcinoma *in situ* in 83 per cent of the cases, a definite invasive growth in 10 per cent and invasio incipiens in 7 per cent.

We believe that the cytologist is not obliged to inform the gynecologist as to whether or not the vaginal smear revealed carcinoma *in situ* or invasive carcinoma since the cytological features of early invasion do not differ from those of carcinoma *in situ*.

Thus, we are of the opinion that whenever the diagnosis of cancer is made by the cytologist, biopsy should be performed and its results must decide the classification of the case.

Olle Kjellgren, Gothenburg, Sweden: I agree with de Brux and colleagues and Campos that in most, but not all, cases we can differentiate between carcinoma *in situ* and immature invasive carcinoma by means of exfoliative cytology.

Wolfgang Korte, Bonn, Germany: Before answering this question one has to answer if an invasive carcinoma can be found by means of exfoliative cytology. Also the pathologist, who does not practice cytology, can affirm this possibility, if one distinguishes between cytological findings and cytological diagnosis.

The cytological findings describe the cellular patterns. The cytological diagnosis may be allowed only to the very expert who can visualize the respective tissue change from which the cells in the smear exfoliated. Strictly speaking, a cytological diagnosis should not be related to tissue changes,

but should be concerned only with changes of cells. In practice we take our definitions from histology and translate them into cytology. This is controversial and may be dangerous.

If one restricts oneself to cytological findings, it is allowable to say: "These cells are changed in such a manner, as is usually found, according to experience, in the smear of an invasive carcinoma." The proof may then be given by the histological diagnosis.

It depends upon the form of cells and tissue, also upon the localization and age of malignant tumors, if and when they desquamate cells. But if cells are desquamated from invasive carcinomas the degree of maturation of the carcinoma determines how conspicuous they are in the smear.

If one may distinguish cells in the smear, which are typical for invasive carcinoma, one should be able to distinguish them from cells which are typical for a so-called carcinoma *in situ*. This will be possible in some cases.

One can answer the original question in the affirmative with reservations. For reasons of method, definition and objectivity one is not able to fully answer this question in the affirmative, especially since the histological examination is also difficult or impossible in borderline cases, according to our experience.

Alexander Meisels, México, D.F., México: De Brux asks the pertinent question: Is the carcinoma *in situ* really an entity? Pathologists all over the world are still discussing this question. They have been unable, so far, to agree even on the main characteristics of the histology of this lesion. Cases are on record that were authoritatively classified as carcinoma *in situ* by some pathologists, and as basal hyperplasia or "cellular atypia" by others. We cytologists are caught in this maze of argument as soon as we mention the words "carcinoma *in situ*." As long as pathologists do not reach an agreement, cytologists will have similar difficulties, unless they try to reach a purely cytologic level of agreement.

The fact that some carcinomas *in situ* remain unchanged for many years seems to point toward some intrinsic biologic characteristics at the cellular level, which account for the lesser degree of aggression, as compared with invasive carcinoma. In cytology, we are accustomed to see morphology reflect even minute biologic changes. It is not surprising, therefore, that cells desquamated from a carcinoma *in situ* have certain morphologic features that may enable the cytologist to differentiate them from those originating in an invasive carcinoma.

Laguna, Meisels, Munguía reported in 1955, at the First Latin-American Pathology Congress, that it is possible, by means of cytology, to distinguish the intra-epithelial or incipient invasive carcinoma from the invasive one, in over 80 per cent of the cases. This was recently confirmed by M. Solis in a review of over 120 cases reported by several cytologists. It is interesting to note that this accuracy does not simply reflect the adjustment of one cytologist to the criteria of one pathologist, since these cases were reported by different cytologists and controlled by various pathologists.

Luis Montalvo-Ruiz, Madrid, Spain: We can study the exfoliative cytology of carcinoma *in situ* and invasive cancer by morphological cytochemical and biochemical methods.

By the morphological method is is very difficult to determine whether a cancer is invasive or not, since the only fundamental criterion, and we agree here with Hopman, is penetration of the basement membrane, which cannot be seen by means of exfoliative cytology.

In some smears the finding of leukocytes and vaginal flora with many necrotic cells can be compatible with invasive carcinoma; that is, we can differentiate by indirect signs more than by morphology. But this does not always happen, as is shown by the two similar photomicrographs. Figure 1 is a carcinoma *in situ* and Figure 2 is an invasive carcinoma, both containing abundant Döderlein bacilli. It has been demonstrated by

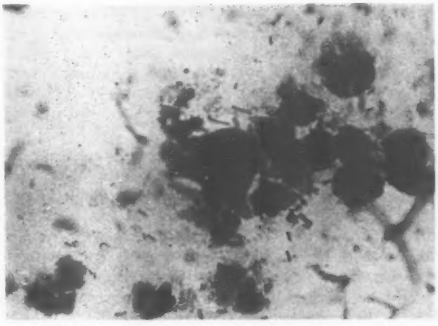

FIG. 1. Carcinoma *in situ*.

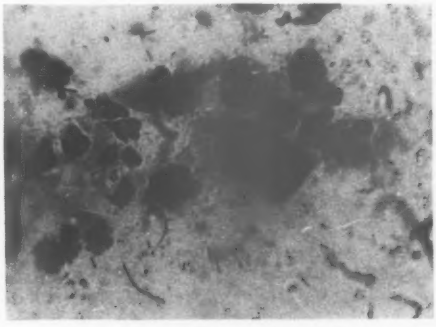

FIG. 2. Invasive carcinoma.

Boschann that the cytochemical qualities of the malignant cells are the same in both *in situ* and invasive carcinoma.

Biochemically, it has been demonstrated by Limburg that the metabolic quotient of anaerobic glycolysis measured by the Warburg apparatus is the same in both *in situ* and invasive carcinoma, the values varying between +21 and +24.6 as distinguished from the benign proliferations with values between +8 and +13.

Navratil believes that a cytological differentiation is not possible between noninvasive and invasive cervical carcinoma. Gompel, in the International Symposium of Exfoliative Cytology of Brussels, stated that the cytologic criteria for malignancy are the same for invasive and noninvasive carcinomas. We agree with this criterion.

Violette M. Nuovo, Paris, France: We agree with de Brux, Campos and Hopman that cytology in most cases is highly suggestive for carcinoma *in situ*, but only suggestive.

In our records we were able to differentiate cytologically approximately 70 per cent of the cases of carcinoma *in situ* from invasive carcinoma.

(1) The cases which were cytologically diagnosed as invasive carcinoma and which were histologically carcinoma *in situ* were already discussed (Acta Cytologica. **3:** 95, 1959).

(2) We had ten cases which were cytologically suggestive for carcinoma *in situ* but which were histologically invasive carcinoma. These cases were all poorly differentiated epidermoid carcinomas.

Therefore our results appear different from those obtained by de Brux, since the mature invasive carcinoma is rather easily distinguished by us, while, in my opinion, it is difficult to differentiate the immature invasive epidermoid carcinoma from a carcinoma *in situ*. The cytomorphological descriptions which de Brux gives in this symposium of the immature invasive carcinoma and the carcinoma *in situ* seem to me very similar, while his descriptions of the cytomorphology of the mature carcinoma and the carcinoma *in situ* seem very different.

How, then, does de Brux conclude that "the immature invasive carcinoma is easily distinguished; it is rather difficult, on the contrary, to differentiate the mature epidermoid carcinoma," or did I misunderstand him?

Concerning the paper by Hopman: Can we be certain that "carcinoma *in situ* represents the early stage of invasive carcinoma"? This lesion seems to be reversible in some cases and we do not know if all invasive carcinomas are preceded by carcinoma *in situ*.

Thomas A. Slate, San Diego, California, U.S.A.: In general, we believe there is a valid correlation between exfoliative cytology, obtained by the cervical smear, and representative histopathologic changes. We have been pursuing this study of correlation for the past five years, and we are convinced that in the majority of cases there are definite cytologic changes that correlate with progressing atypical histopathologic changes.

We agree with de Brux and Dupré-Froment that cytologically it is very difficult to distinguish between marked dysplasia and early carcinoma *in situ*, and also at the other end of the scale, to distinguish between extensive or late carcinoma *in situ* and early invasive carcinoma. It is understood by all that the final diagnosis still must rest with the histologic findings, but it is of added assurance to the clinician or gynecologist if the cytologist indicates, when possible, whether the cells are suggestive of a dysplasia, carcinoma *in situ*, or invasive carcinoma.

During 1958 we reported 120 Class IV smears in which we suspected carcinoma *in situ*. Histologic follow up revealed 12.5 per cent invasive carcinoma and the remainder were either carcinoma *in situ* or lesser atypias. To date, during 1959, 13 per cent of our Class IV smears which suggested carcinoma *in situ* have revealed invasive carcinoma.

A. I. Spriggs, Oxford, England, U.K.: Like Hopman, we have made a comparative study of "positive" smears and the corresponding biopsies (Brit. J. Cancer. **14:** 151, 1960). The smears were reexamined (without identification marks) by myself and Mr. M. M. Boddington, and the histological slides by Dr. R. H. Cowdell. Six cytological groups and 11 histological groups were distinguished; the latter included abnormalities from basal cell hyperplasia through early well-differentiated carcinoma up to the most anaplastic form. In each histological grade there was a scatter of cytological gradings around a modal peak. Although the degree of differentiation of the superficial cells was shown in the smears, there was not the slightest evidence that the onset of invasion necessarily coincides with a change in morphological cell type.

This accords well with the view that carcinoma develops by a series of discontinuous transformations, occurring probably at random once the process has begun, and that invasion is an incident which may arise at any time and not in association with a definite morphological stage. We can see in exfoliated cells all the changes from normal up to the most anaplastic forms, the latter being usually associated with advanced cancer; we cannot, however, determine from smears what is going on underneath.

L. A. Turnbull, Montreal, Canada: During the past ten years of our study concerning preclinical carcinoma of the cervix, our gyne-cytology laboratory of the Royal Victoria Hospital has been very closely associated with our gyne-pathology department, and seeing both sides of this question, we have come to the conclusion that carcinoma *in situ* could be, with a high degree of accuracy, differentiated by cytology from invasive carcinoma of the cervix. However, we still depend greatly upon our

pathological diagnosis, bearing in mind our cytological opinion.

I agree with Dupré-Froment in his description of the details of the nuclear and cellular changes which the cytologist is able to observe in smears and thus differentiate a carcinoma *in situ* of the cervix from a late malignant degeneration of the cells and their nuclei.

The one thing that is dangerous about this is the recognition of the cases with early invasion.

In the Women's Pavilion of the Royal Victoria Hospital, Montreal, with the establishment of extensive cytological screening, all patients who are diagnosed by cytology as positive for cancer are undergoing biopsy and further treatment. Cytologically we divide all preclinical cancer cases into three groups, corresponding to our pathological pictures.

Cytology		*Pathology*
Grade 3A	→	carcinoma *in situ*
Grade 3B	→	early invasive carcinoma
Grade 3C	→	invasive carcinoma

By having the pathological picture on each of the cytologically diagnosed cancer cases and comparing them to cytological smears, we are able to see our accuracy in cytological grading.

Judging by our statistics we are able to present such figures.

1. In our Grade 3A, or carcinoma *in situ*, we have about 85 per cent of accurately diagnosed cases by cytology; about 6 per cent were diagnosed by pathology as early invasive carcinoma and about 9 per cent as metaplastic or anaplastic benign smears.
2. In Grade 3B, as early invasive carcinoma, we found 76 per cent early invasive carcinoma, 10 per cent carcinoma *in situ*, and 14 per cent invasive preclinical carcinoma of the cervix.
3. In Grade 3C, we, as a rule, found invasive or even clinical carcinoma of the cervix with just a small percentage of error due to overstained smears or due to the smear being taken following a previous manipulation of the cervix.

Hans-Klaus Zinser, Cologne, Germany: In the discussion of the topic "Advantages, Disadvantage and Diagnostic Accuracy of Differentiating Dysplasia, Carcinoma *in Situ* and Invasive Cervical Carcinoma by Means of Exfoliative Cytology," I already have given some of our results to this question. I can only agree with the opinions of the main authors and I am not able to add any essentially new viewpoints. We can confirm that cytologically the demarkation of carcinoma *in situ* from metaplastic lesions is very difficult. However, this occurred in only three of 72 cases which we reported cytologically as carcinoma *in situ*. Much more frequently we have reported small carcinomas as carcinoma *in situ:* seven of the 72 cases. Without question the degree of maturation plays an important role in this false evaluation of the cytological picture. It has been stated several times, however, that in such cases histology shows, in addition to a small localized carcinoma, a carcinoma *in situ* and thus the cytological misinterpretation becomes explainable. Generally speaking it seems to me that the cytological prediction of carcinoma *in situ* is not essentially safer than the diagnosis of genuine carcinoma, especially if we include the neoplastic lesions of minimal extent.

Closing Remarks

Jean de Brux: Some of the discussants have anticipated and answered the questions formulated by other discussants. Thus, Meisles has answered completely Anderson's astonishment at my question, "Is carcinoma *in situ* really an entity?" Ruth Graham and Slate replied for me to Violette Nuovo. Nevertheless, I wish particularly to state the following:

1. The differences encountered in cytology as well as in histology are due essentially to the fact that there exist no criteria universally accepted by pathologists.

2. Hence, it is difficult to distinguish with precision the irregular dysplasias, the atypical hyperplasias of the reserve cells, and carcinoma *in situ*. These three lesions nevertheless have histological criteria, rather slender, but sufficient to permit their differentiation.

If there is a doubt, the cytological criteria intervene to aid in differentiation. In the dysplasias, dyskaryotic and dysplastic elements of parabasal type are found. In the atypical hyperplasia of the reserve cells (active, undifferentiated and immature metaplasia), there exist particular elements the description of which we have given in another topic of the Symposia. The carcinoma *in situ* is rich in elements which we have described under the term of carcinoma *in situ* cells, exfoliated as isolated cells.

Under these conditions, one cannot say that, among the major criteria, there is "first of all a feeling that cannot be translated into words." Cytology must be directed by a severe discipline, otherwise it will fall into the category of "kitchen recipes." We must therefore profit by our mistakes, and, by comparing the pathological specimen with the smear, try to establish cellular types as precisely as possible, corresponding to distinct lesions. In so far as the irregular dysplasias and the

active, undifferentiated and immature metaplasias are concerned, we have succeeded in this effort. As for the carcinoma *in situ*, we now strictly reserve this diagnosis for those cases in which we believe the lesion will invade. The cells and the pathological specimens in this case have a special aspect which we are now trying to define sharply before publication.

3. As to the apparent contradictions found by Violette Nuovo in my paper, the following may clarify my thought: When there exists a so-called carcinoma *in situ* (in reality an irregular dysplasia), exemplified classically on the smear by the presence of cornified elements with dysplastic and dyskaryotic nuclei, it may be rather difficult to differentiate it from a very mature invasive epidermoid carcinoma exfoliating only to a slight degree. Where there is a "true" carcinoma *in situ*, clearly characterized by elements such as we have described elsewhere in this issue, the differential diagnosis which may be made is that of invading immature carcinoma. In the latter, however, the cells are much less well preserved, often cytolyzed, and, above all, exfoliate in shreds, with inflammatory and hemorrhagic phenomena, whereas in the "true" carcinoma *in situ* the elements are isolated and only rarely show signs of maturation.

Jorge Campos R. de C.: I would like to refer to Anderson's comments. From the point of view of the gynecologist's routine work, Anderson is probably correct when he says that it is unnecessary to distinguish, cytologically, carcinoma *in situ* from infiltrating carcinoma, because in either case we must wait for the histological diagnosis. However, from the point of view of the cytologist, and for the future progress of cytology, it is very important to find out if there is any difference between cells which come from lesions with different histologies.

B. Cornelis Hopman: Although it is not always possible to make an exact cytologic diagnosis of the stage of the malignant process, the mentioned characteristics give some definite insight into this matter. We should not forget that histology also has its limitations in grading cervical cancer. Not only do pathologists differ in opinion on borderline cases, but the only definite pathologic diagnosis in cervical malignancy is invasive cancer. A diagnosis of carcinoma *in situ* always leaves the question of whether or not an invasive process still exists, due to insufficiency of the material examined.

I agree with Bajardi that a diagnosis should not be based solely on exfoliative cytology. I concur with Boschann that a cytologic differentiation would not relieve the clinician of histologic confirmation.

Ferreira bases her diagnosis mostly on the degree of differentiation of cells, with which I fully agree. If the cytologic slides show no malignancy further advanced than dyskaryotic cells, an invasive cervical cancer is uncommon. Yet, according to the P.C.R. (Positive Cytology Registry of Dr. Ferguson, Chairman of the Dept. of Obstetrics and Gynecology, Miami School of Medicine), invasive cervical cancers occur in 14 per cent of Papanicolaou Class III malignancies.

I agree with Graham that cancer cells are frequently found in advanced cervical cancer. However, in the very far advanced fungating, crater or cauliflower form of malignancy the cancer cells may be missed by cytology. Due to extensive infection and bleeding the whole slide is covered with white and red blood cells, so that the small dedifferentiated malignant cells may be difficult to find. Besides that they are often seen only in small numbers, being rinsed away by blood and exudate. These cases show cells in a stage further advanced than that we usually refer to as Class V (Papanicolaou).

I don't agree with Maria Kawecka and Hanna Starkiewicz that cytology is ever unnecessary in cervical cancer diagnosis even in an advanced case. Cytology should always be done because the biopsy may consist of necrotic tissue only, even if a target lesion is present. I agree that in general, histology must decide the classification of the case.

I agree with Kjellgren that the cases of carcinoma *in situ* and undifferentiated invasive cancer can readily be recognized by cytology, in most cases.

In reply to Nuovo: the question whether or not carcinoma *in situ* represents an early stage of invasive carcinoma is answered in the affirmative, although proof is difficult to realize. To diagnose carcinoma *in situ* one must remove the lesion and to prove that it is an early stage of invasive cancer one must leave it in. This is of course impossible. However, many factors such as deviations appearing in the same histologic preparation with distinct transitions; age differences of both deviations in patients; frequency differences in various races; etc., make it highly probable that carcinoma *in situ* really represents the early stage of invasive cancer. This whole issue is described in detail by Hertig in a publication "What is cancer *in situ*? Is it the Preinvasive Stage of True Carcinoma?" (Am. J. Obst. & Gyn. **64**: 807, 1952.) That carcinoma *in situ* could seem to be reversible does not preclude it to be an early stage of invasive cancer. Cancer very probably may revert to normal at any stage. However, the further the malignant process has developed the less likely it is to revert to normal.

Abstracts

Cytological diagnosis of mixed tumors of the salivary glands (Russian Text). Nikitina, N. I. Vopr. Onkol. 7(2): 43-48, 1961.

THE PUNCTATES are spread delicately between two glass slides and stained according to the Pappenheim method or with hemotoxylin and eosin. In 35 cases cytological and histological examinations were performed. Cytologically, the tumors are divided into three groups differing in the kind and distribution of the cells. The author states that mixed tumors may be recognized by their cytological pattern, but that their transition into carcinoma is often difficult to detect. In contrast to histology, cytology does not make it possible to differentiate mixed tumors from cylindromas.

The differential diagnosis of inflammation and carcinomatous infiltrate in treated cervical carcinoma (Zur Differentialdiagnose von entzündlichem und carcinomatösem Infiltrat des behandelten Collum-ca). Wennemann, J. Ges. z. Bek. d. Krebskrankheiten, Düsseldorf. 2(2): 3-7, 1960.

AFTER mentioning the difficulties inherent in differential diagnosis in the identification of the parametrial infiltrates and the therapy of cervical carcinoma, the author reports on his experiences treating 30 patients with Prednisolone. At the end of therapy one can differentiate irradiative inflammation from carcinomatous infiltrates. This type of therapy is recommended for the verification of diagnoses because it is not yet known whether antipholgistic hormones have a negative effect on tumor growth in the human. The author suggests that the potential diagnostic procedure will improve the relative cure rate of carcinomatous infiltrative infection.

Is the early detection rate of cervical cancer being maintained? (Hält die Früherfassung des Collum-ca an?). Wenneman, J. Ges. z. bek. d. Krebskrankheiten, Düsseldorf. 2 (3-4): 3-11, 1961.

THE CHANGE in the relative composition of the stages of detected cases of cervical cancer during the years 1952 to 1955 (relative increase of Stage I and relative decrease of Stage II) is neither a coincidence nor the result of specially selected patient material. Among 622 cases of cervical cancer detected in those years grouped according to the usual stages, 54.7 per cent belonged to Stage I, 26.1 per cent to Stage II, 15.8 per cent to Stage III, and 3.4 per cent to Stage IV. There is a slight decrease in the number of Stage III lesions detected. In 1960 for 153 patients with cancer of the cervix, Stages I through IV, there were 11 patients with Stage 0 lesions. This demonstrates clearly the efficiency of the publicity campaign of the Society for the Fight against Cancer of the State North Rhine Westfalen of Germany in educating the public and promoting the early diagnosis of cancer.

The cytological pattern in cases with a history of overmaturity (Das cytologische Bild bei Fällen von anamnestischer Übertragung). Jenny, J. Gynaecologia. 151(3): 174-184, 1961.

CERTAIN easily detectable cytological changes appearing during the last week before partus allow the determination of impending labor. Smears taken in late pregnancies show little signs of readiness for labor so that the confinement is generally not to be expected within the next few days and the medical induction of labor will usually fail. This is different in smears of the last week and especially at term with a positive ovocyclin test. The cytological picture allows also conclusions as to the functions of the placenta without, however, the possibility of determining an intra-uterine asphyxia. No typical cytological changes of postmaturity could be found. One can, however, assume that with a late pregnancy smear no signs of postmaturity in the child would be detected. With the other two types of smears a biological postmaturity is possible but by no means certain. Even the smear at term with regressive changes is neither characteristic of postmaturity nor of impending danger for the baby.

The evaluation and comparative examination of stained and vital vaginal specimens (Beurteilung und Gegenüberstellung der Auswertungsergebnisse der Vaginalzytologie anhand des gefärbten des Vitalpräparates). Delnon, I. Gynaecologia. 151(1): 1-18, 1961.

THE DAILY experience with gynecological cytology demonstrates that objective evaluation without a subjective way of observation is not possible. The reason for this lies in the inadequacy of a morphological method *per se* and the lack of adequate criteria respectively. Some important diagnostic factors, which need exploration, are mentioned. The vaginal cytological picture is determined by a number of interrelated endo- and exogenic procedures only a part of which is known. On the basis of several examples the difficulty of interpretation, which may occasionally arise, is demonstrated. The certainty of a vital or stained specimen in the explanation of functions and

recognition of atypical cells, is equal for the experienced investigator. Apart from the morphological examination of exfoliated cells, biochemistry will search for cytochemical methods to improve the results. Vaginal cytology is considered a clinical method which should answer the questions of the practicing gynecologist.

Cytological and histological studies on the atypical transformation zone (Zytologische und histologische Untersuchungen zur atypischen Umwandlungszone). Wagner, D. and Fettig, O. Geburtshilfe und Frauenheilkunde. 21(2): 156-169, 1961.

1. In 80 per cent of cases with an atypical transformation zone on colposcopy, vaginal smears, obtained by scraping the portio vaginalis of the cervix were negative. The results of histological and cytological examination were identical in 95 per cent of these cases.

2. In all cases, in which positive vaginal smears were obtained from colposcopically atypical transformation zones, either carcinoma *in situ* or invasive carcinoma was revealed histologically. The index of malignancy in our cases with atypical transformation zones was 13.6 per cent, which corresponds with the index of malignancy (13.8 according to Burghardt) for the classical area of origin of carcinoma of the cervix (Matrixbezirke).

3. In the 5.5 per cent of cases with an atypical transformation zone the cytological findings were doubtful (Papanicolaou III), and in such cases histological sections showed abnormal regenerative changes, which demanded continued observation of the lesion.

4. There were two false negative smears which showed an abnormally active epithelium on histological study; these were attributed to observer error in one case and to error of technic in the other.

5. In 14 of the 44 (30 per cent) negative smears histological evaluation of the epithelium was not possible as it was missing, in some cases because of a true erosion and in others as the result of artifacts caused by taking the biopsies. The state of the epithelium in these cases was therefore assessed by cytology only.

The results show that cytological examination enables transformation zones, which colposcopically are not definitely benign, to be classified cytologically as benign or as atypical transformation zones. By routine cytological examination of smears from colposcopically doubtful areas it is possible to limit biopsy of the cervix to those patients with either positive or doubtful smears.

The importance of colposcopic examination (Die Bedeutung der kolposkopischen Triage). Schreiner, W. E. and Abbuhl, A. Gynaecologia. 151(3): 203-211, 1961.

In addition to former investigations of 1,413 originally colposcopic nonsuspicious patients, 731 cases could be further examined colposcopically and cytologically. 9.3 per cent of them showed colposcopically suspicious findings and were further investigated by biopsy, curettage, and ringbiopsy. Altogether three cases of cancer *in situ* and two early invasive cancers of the cervix were discovered in clinically symptomless patients giving an incidence of 6.8 per cent (4.1 0/00 Stage 0 and 2.7 0/00 Stage Ia). These figures are compared with the incidence rate of a colposcopically suspicious group of the same material and the incidence or prevalence rate of those originally obstetric patients is compared with a gynecological or average population.

On the basis of these investigations the importance of colposcopic and cytological examination for an early lesion of symptomless cancer of the cervix is emphasized.

Forthcoming Meeting

New York Academy of Sciences Conference on
THE CERVIX
December 7, 8, and 9, 1961
The Henry Hudson Hotel
New York City
Conference Co-chairmen:
Warren R. Lang, M.D.
Jefferson Medical College, Philadelphia, Pennsylvania
Alfred B. Kupferberg, Ph.D.
Ortho Research Foundation, Raritan, New Jersey

Correspondence

To the Editors:

The discussions and controversies about carcinoma *in situ* continue, and not only are there controversies of interpretations of individual histological sections but also controversies regarding the criteria for the histological diagnosis of carcinoma *in situ* and upon this the statistics for the incidence of carcinoma *in situ* in cytologic screening are based. Since the criteria for the pathological diagnosis of carcinoma are based entirely on the interpretation of the static morphological manifestations, it is a limited point of view and thereby has limitations that may be preventing us from perceiving the essence of carcinoma in its complete dynamic dimensional evolvement. Of course carcinoma has the three dimensions of any tangible object but is also an expanding mass of cells in motion. There are topological considerations.

It may be well to consider the sequence of events of the normal dynamic cervical epithelium of the child-bearing era in which a basal cell multiplies to form two cells, one parabasal cell and one basal cell. The parabasal cell multiplies to form a variable number of generations which become stratified toward the surface. Then by means of some governing control the parabasal cells lose their ability to divide and start their differentiation toward the superficial keratinized cell through the intermediate cell stage. In this normal sequence of events there are several obvious processes that are going on. The cellular division is taking place by mitoses that are in a plane that is parallel to the basement membrane. There is a well governed number and/or rate of mitoses. There is some governing influence that determines which of the daughter cells is to cease multiplying and mature and which is to stay on as a reserve cell. Obviously, if all cells were to mature eventually the epithelium would entirely exfoliate and only a denuded surface would remain. If neither of the daughter cells were to mature and continue to multiply then we would have immature cells making up the entire thickness of the epithelium and we would have a morphologically equivalent picture of reserve cell hyperplasia and approach the appearance of carcinoma *in situ*.

The mitotic plane of the proliferating cells requires consideration because if it were other than parallel to the basement membrane, cells would not be migrating in the orderly stratification toward the surface that is normally seen. A loss of the force that governs the mitotic plane of proliferating cells would result in cellular masses moving in any and all directions. If the mitoses resulted in cells moving sideways this would upset the normal migratory movement of adjoining cells and a turbulent cytological pattern would be produced where these two cytologic streams would collide. Is this not the pattern one sees in carcinoma? If the proliferating cells had mitotic planes that rotated 180 degrees the cells may migrate away from the surface and push into the basement membrane and give us the histological picture of leukoplakia or rupture through the basement membrane and give us the picture we see with invasive carcinoma. The invasiveness of a carcinoma could be due to the invasive nature of a cancer cell or due to the loss of the integrity of the basement membrane or due to both factors. Maybe carcinoma will spread peripherally until a weak spot is met in the basement membrane. All of us have seen what appears to be an early carcinoma that is already invasive and have seen larger and perhaps older carcinomas that have not invaded. This variable picture could be determined by the integrity, permeability or strength of the basement membrane as opposed to the variable force of expanding malignant cellular mass extending along cleavage planes to a variable degree depending upon the resistance offered by different tissue structural barriers.

Hence, the picture of the normal cervix is the resultant of two governing forces, *viz.*, forces that govern the onset of cellular maturation and the forces that govern the polarity of mitoses or nuclear polarity. Loss of the first can lead to a reserve cell hyperplasia and loss of the second can lead to disorderly array of cells growing in all directions at random. Conceivably or perhaps admittedly loss of all governing forces leads to carcinoma.

It may be expedient for our comprehension to think of carcinoma as starting with one cell and since it has to start in a cell capable of dividing it would have to start in a basal cell or parabasal cell. Such cells are not normally at the surface so these cells cannot exfoliate. If such cells were at the surface as could occur with reserve cell hyperplasia and if it did exfoliate there would be spontaneous cure. Perhaps this occurs in those cases that remit after pregnancy.

For purposes of this discussion it would be best to consider the carcinomatous cell located in the basal layer or parabasal layer. If it were a cell in the basal layer or parabasal layer this

carcinomatous cell would divide to give rise to two or more carcinoma cells and in the process of this division the parent cell would cease to exist because it would now be represented by the two daughter cells. These two cells would be dividing randomly in all planes and forming a cellular mass that would expand toward the surface. These malignant cells cannot exfoliate until the surface is reached. The stratified squamous epithelium of the cervix is of variable thickness but for the sake of discussion we can consider the epithelium to be 20 cells thick. If we consider a malignant cell as being totally ungoverned with regards to the plane of mitoses and therefore multiplying randomly in all planes it will tend to form a morula 20 cells in diameter before it reaches the surface.

It is most important to realize that cells cannot exfoliate if they are buried. A cluster of carcinoma cells growing beneath an overlying stratum of normal cells cannot manifest itself in cytological smears. Any biopsy revealing a suspicious cluster of cells that does not extend to the surface cannot be utilized to explain the presence of malignant or suspicious cells in a smear. Of course before this statement can be made with assurance one must have serial sections of the entire lesion.

It would seem that as a mass of malignant cells are growing in the deeper layers of the cervical epithelium the overlying relatively normal epithelium undergoes aberrations that resemble some of the features of malignant tissue in that they are being pushed into disarray and their nuclei, although they are not the picture of malignancy, have slight aberrations that could be due to the diffusion of some carcinogenic influence from below or maybe the physical force exerted from below disturbs the physicochemical nuclear reactions or cellular nutrition. Such a histologic picture would conform to that of cervical dysplasia and the cells exfoliating from such a surface would be those that are identified as dyskaryotic.

Whether or not this truly represents an early carcinoma is a moot point but if it is carcinoma it is cured by biopsy and for practical purposes it may be just as well to diagnose it as cervical dysplasia. The subsequent appearance of carcinoma in the same cervix would imply a new independent growth in a cervix that has a predilection to grow carcinoma. For pragmatic purposes carcinoma *in situ* should not be diagnosed until the full thickness of the epithelium is involved because it cannot exfoliate its cells until it does emerge although it may cause accelerated exfoliation of the overlying epithelium that has the altered appearance of dyskaryosis.

If by definition a carcinoma *in situ* has to involve full thickness of the epithelium its minimal size as viewed by the naked eye or by colposcopy would have to be the same as or of greater diameter than the thickness of the epithelium since it is presumed that carcinoma grows equally in all directions unless the epithelium superficial to the carcinoma has sloughed off. In this way a cervical dysplasia could be converted to a carcinoma *in situ*, but such a happening would allow exfoliation of cancer cells and could explain malignant cytologic smears that would not occur without this exposure to the surface. If one admits the above premise then one would conclude that the diameter of a carcinoma *in situ* cannot be less than the thickness of its epithelium or about 0.3 to 0.5 mm. in diameter.

The writer finds it disturbing to see pictures published in the journals that exhibit carcinomas *in situ* that have not extended to the surface and cannot explain the exfoliated malignant cells. The presence of malignant cells in an exfoliated smear may serve to prove that the carcinoma *in situ* had emerged to the surface but in most instances the findings are used to prove the malignant nature of the exfoliated cells. This is obviously faulty logic wherein a hypothesis is being proved by itself; *viz.*, there is histological carcinoma *in situ* because there are malignant exfoliated cells because there is a carcinoma *in situ*.

It would seem to be most imperative that cytologists refrain from proving cells as malignant until the area of exfoliation is histologically demonstrable at a surface. Once this is proved then any subsequent identification of the exfoliated cell will serve to identify it as malignant. This will serve to define precisely the cytologic criteria for the identification of malignancy and it will eliminate the aberrant cells that are being influenced by the neighboring malignant cells from being included in the cytologic pattern of malignancy and putting them in the category of near malignancy or neomalignant cells. The writer believes that neocancer cells are not near cancer in their evolvement but near cancer because of proximity to cancer cells and are being influenced by the proximity to or by actual inclusion in an abnormal cellular milieu.—F. X. MacAulay, M.D., Director of Laboratories, Orangeburg Regional Hospital, Orangeburg, South Carolina.

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